



Z'-LYTE® Screening Protocol and Assay Conditions

Revised 15-Dec-2010

Page 1 of 35

**ASSAY THEORY** \_\_\_\_\_ **3**

**Z'-LYTE® ASSAY CONDITIONS** \_\_\_\_\_ **4**

**Z'-LYTE® ASSAY CONTROLS** \_\_\_\_\_ **5**

**Z'-LYTE® DATA ANALYSIS** \_\_\_\_\_ **6**

**KINASE-SPECIFIC ASSAY CONDITIONS - DIRECT FORMAT** \_\_\_\_\_ **7**

ABL1	7	EGFR (ErbB1) T790M	12	MAP4K2 (GCK)	17	PRKCB1 (PKC beta I)	23
ABL1 E255K	7	EGFR (ErbB1) T790M L858R	12	MAP4K4 (HGK)	18	PRKCB2 (PKC beta II)	23
ABL1 G250E	7	EPHA1	12	MAP4K5 (KHS1)	18	PRKCD (PKC delta)	23
ABL1 T315I	7	EPHA2	12	MAPK1 (ERK2)	18	PRKCE (PKC epsilon)	23
ABL1 Y253F	7	EPHA4	12	MAPK11 (p38 beta)	18	PRKCG (PKC gamma)	23
ABL2 (Arg)	7	EPHA5	12	MAPK12 (p38 gamma)	18	PRKCH (PKC eta)	23
ACVR1B (ALK4)	7	EPHA8	13	MAPK13 (p38 delta)	18	PRKCI (PKC iota)	23
ADRBK1 (GRK2)	7	EPHB1	13	MAPK14 (p38 alpha) Direct	18	PRKCN (PKD3)	24
ADRBK2 (GRK3)	7	EPHB2	13	MAPK3 (ERK1)	18	PRKCQ (PKC theta)	24
AKT1 (PKB alpha)	7	EPHB3	13	MAPKAPK2	18	PRKCZ (PKC zeta)	24
AKT2 (PKB beta)	7	EPHB4	13	MAPKAPK3	18	PRKD1 (PKC mu)	24
AKT3 (PKB gamma)	8	ERBB2 (HER2)	13	MAPKAPK5 (PRAK)	18	PRKD2 (PKD2)	24
AMPK A1/B1/G1	8	ERBB4 (HER4)	13	MARK1 (MARK)	19	PRKG1	24
AMPK A2/B1/G1	8	FER	13	MARK2	19	PRKG2 (PKG2)	24
AURKA (Aurora A)	8	FES (FPS)	13	MARK3	19	PRKX	24
AURKB (Aurora B)	8	FGFR1	13	MARK4	19	PTK2 (FAK)	24
AURKC (Aurora C)	8	FGFR2	13	MATK (HYL)	19	PTK2B (FAK2)	24
AXL	8	FGFR3	14	MELK	19	PTK6 (Brk)	24
BLK	8	FGFR3 K650E	14	MERTK (cMER)	19	RET	25
BMX	8	FGFR4	14	MET (cMet)	19	RET V804L	25
BRSK1 (SAD1)	8	FGR	14	MET M1250T	19	RET Y791F	25
BTX	8	FLT1 (VEGFR1)	14	MINK1	19	ROCK1	25
CAMK1D (CaMKI delta)	8	FLT3	14	MKNK1 (MNK1)	19	ROCK2	25
CAMK2A (CaMKII alpha)	9	FLT3 D835Y	14	MST1R (RON)	20	ROS1	25
CAMK2B (CaMKII beta)	9	FLT4 (VEGFR3)	14	MST4	20	RPS6KA1 (RSK1)	25
CAMK2D (CaMKII delta)	9	FRAP1 (mTOR)	14	MUSK	20	RPS6KA2 (RSK3)	25
CAMK4 (CaMKIV)	9	FRK (PTK5)	15	MYLK2 (skMLCK)	20	RPS6KA3 (RSK2)	25
CDC42 BPA (MRCKA)	9	FYN	15	NEK1	20	RPS6KA4 (MSK2)	25
CDC42 BPB (MRCKB)	9	GRK4	15	NEK2	20	RPS6KA5 (MSK1)	25
CDK1/cyclin B	9	GRK5	15	NEK4	20	RPS6KA6 (RSK4)	25
CDK2/cyclin A	9	GRK6	15	NEK6	20	RPS6KB1 (p70S6K)	26
CDK5/p25	9	GRK7	15	NEK7	20	SGK (SGK1)	26
CDK5/p35	9	GSK3A (GSK3 alpha)	15	NEK9	20	SGK2	26
CHEK1 (CHK1)	9	GSK3B (GSK3 beta)	15	NTRK1 (TRKA)	20	SGKL (SGK3)	26
CHEK2 (CHK2)	10	HCK	15	NTRK2 (TRKB)	21	SNF1LK2	26
CLK1	10	HIPK1 (Myak)	15	NTRK3 (TRKC)	21	SRC	26
CLK2	10	HIPK2	15	PAK1	21	SRC N1	26
CLK3	10	HIPK3 (YAK1)	15	PAK2 (PAK65)	21	SRMS (Srm)	26
CSF1R (FMS)	10	HIPK4	16	PAK3	21	SRPK1	26
CSK	10	IGF1R	16	PAK4	21	SRPK2	26
CSNK1A1 (CK1 alpha 1)	10	IKBK (IKK beta)	16	PAK6	21	STK22B (TSSK2)	26
CSNK1D (CK1 delta)	10	IKBKE (IKK epsilon)	16	PAK7 (KIAA1264)	21	STK22D (TSSK1)	26
CSNK1E (CK1 epsilon)	10	INSR	16	PASK	21	STK23 (MSSK1)	27
CSNK1G1 (CK1 gamma 1)	10	INSRR (IRR)	16	PDGFRA (PDGFR alpha)	21	STK24 (MST3)	27
CSNK1G2 (CK1 gamma 2)	10	IRAK4	16	PDGFRA D842V	21	STK25 (YSK1)	27
CSNK1G3 (CK1 gamma 3)	11	ITK	16	PDGFRA T674I	22	STK3 (MST2)	27
CSNK2A1 (CK2 alpha 1)	11	JAK1	16	PDGFRA V561D	22	STK4 (MST1)	27
CSNK2A2 (CK2 alpha 2)	11	JAK2	16	PDGFRB (PDGFR beta)	22	SVK	27
DAPK3 (ZIPK)	11	JAK2 JH1 JH2	16	PDK1 Direct	22	TAOK2 (TAO1)	27
DCAMK2 (DCK2)	11	JAK2 JH1 JH2 V617F	17	PHKG1	22	TBK1	27
DNA-PK	11	JAK3	17	PHKG2	22	TEK (Tie2)	27
DYRK1A	11	KDR (VEGFR2)	17	PIM1	22	TXK	27
DYRK1B	11	KIT	17	PIM2	22	TYK2	27
DYRK3	11	KIT T670I	17	PKN1 (PRK1)	22	TYRO3 (RSE)	28
DYRK4	11	LCK	17	PLK1	22	YES1	28
EEF2K	12	LTK (TYK1)	17	PLK2	22	ZAP70	28
EGFR (ErbB1)	12	LYN A	17	PLK3	23		
EGFR (ErbB1) L858R	12	LYN B	17	PRKACA (PKA)	23		
EGFR (ErbB1) L861Q	12	MAP3K9 (MLK1)	17	PRKCA (PKC alpha)	23		



**KINASE-SPECIFIC ASSAY CONDITIONS - CASCADE FORMAT** \_\_\_\_\_ **29**

BRAF	29	MAP2K2 (MEK2)	29	MAPK10 (JNK3)	29	MAPK9 (JNK2)	30
BRAF V599E	29	MAP2K6 (MKK6)	29	MAPK14 (p38 alpha)	29	PKD1	30
MAP2K1 (MEK1)	29	MAP3K8 (COT)	29	MAPK8 (JNK1)	29	RAF1 (cRAF) Y340D Y341D	30

**TABLE OF KINASE ATP KM BINS AND INHIBITOR VALIDATION** \_\_\_\_\_ **31**

## Assay Theory

The Z'-LYTE® biochemical assay employs a fluorescence-based, coupled-enzyme format and is based on the differential sensitivity of phosphorylated and non-phosphorylated peptides to proteolytic cleavage (Figure 1). The peptide substrate is labeled with two fluorophores—one at each end—that make up a FRET pair.

In the primary reaction, the kinase transfers the gamma-phosphate of ATP to a single tyrosine, serine or threonine residue in a synthetic FRET-peptide. In the secondary reaction, a site-specific protease recognizes and cleaves non-phosphorylated FRET-peptides. Phosphorylation of FRET-peptides suppresses cleavage by the Development Reagent. Cleavage disrupts FRET between the donor (i.e., coumarin) and acceptor (i.e., fluorescein) fluorophores on the FRET-peptide, whereas uncleaved, phosphorylated FRET-peptides maintain FRET. A ratiometric method, which calculates the ratio (the Emission Ratio) of donor emission to acceptor emission after excitation of the donor fluorophore at 400 nm, is used to quantitate reaction progress, as shown in the equation below.

$$\text{Emission Ratio} = \frac{\text{Coumarin Emission (445 nm)}}{\text{Fluorescein Emission (520 nm)}}$$

A significant benefit of this ratiometric method for quantitating reaction progress is the elimination of well-to-well variations in FRET-peptide concentration and signal intensities. As a result, the assay yields very high Z'-factor values (>0.7) at a low percent phosphorylation.

Both cleaved and uncleaved FRET-peptides contribute to the fluorescence signals and therefore to the Emission Ratio. The extent of phosphorylation of the FRET-peptide can be calculated from the Emission Ratio. The Emission Ratio will remain low if the FRET-peptide is phosphorylated (i.e., no kinase inhibition) and will be high if the FRET-peptide is non-phosphorylated (i.e., kinase inhibition).

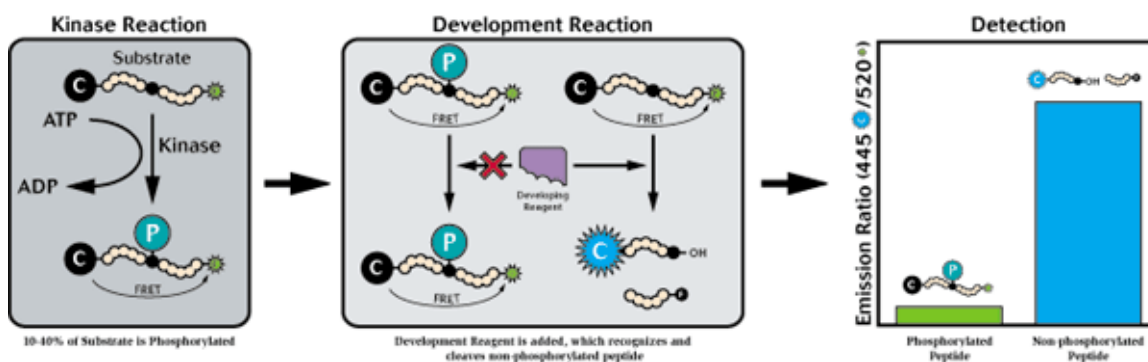


Figure 1: Z'-LYTE® Illustration



## Z'-LYTE<sup>®</sup> Assay Conditions

### Test Compounds

The Test Compounds are screened in 1% DMSO (final) in the well. For 10 point titrations, 3-fold serial dilutions are conducted from the starting concentration of the customer's choosing.

### Peptide/Kinase Mixtures

All Peptide/Kinase Mixtures are diluted to a 2X working concentration in the appropriate Kinase Buffer (see section *Kinase Specific Assay Conditions* for a complete description).

### ATP Solution

All ATP Solutions are diluted to a 4X working concentration in Kinase Buffer (50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA).

ATP Km apparent is previously determined using a Z'-LYTE<sup>®</sup> assay.

### Development Reagent Solution

The Development Reagent is diluted in Development Buffer (see section *Kinase-Specific Assay Conditions - Direct and Cascade* for a complete description).

**10X Novel PKC Lipid Mix:** 2 mg/ml Phosphatidyl Serine, 0.2 mg/ml DAG in 20 mM HEPES, pH 7.4, 0.3% CHAPS

#### For 5 mL 10X Novel PKC Lipid Mix:

1. Add 10 mgs Phosphatidyl Serine (Sigma Part # P6641) and 1 mg DAG (Avanti Polar Lipids PV # N2004) to a glass tube.
2. Remove the chloroform from lipid mixture by evaporation under a stream of nitrogen.
3. Add 5 mLs resuspension buffer, 10% CHAPS / 500 mM HEPES, pH 7.4, to the dried lipid mix
4. Heat gently to 50-60 °C and Vortex in short intervals until the lipids are dissolved and the solution clears. The dissolution of the lipids may take several hours; sonication in a water bath may hasten this process.
5. Aliquot into single use volumes and store at – 20 °C.

### Assay Protocol

*Bar-coded Corning, low volume NBS, black 384-well plate (Corning Cat. #3676)*

1. 2.5 µL – 4X Test Compound or 100 nL 100X plus 2.4 µL kinase buffer
2. 5 µL – 2X Peptide/Kinase Mixture
3. 2.5 µL – 4X ATP Solution
4. 30-second plate shake
5. 60-minute Kinase Reaction incubation at room temperature
6. 5 µL – Development Reagent Solution
7. 30-second plate shake
8. 60-minute Development Reaction incubation at room temperature
9. Read on fluorescence plate reader and analyze the data



## Z'-LYTE® Assay Controls

The following controls are made for each individual kinase and are located on the same plate as the kinase:

### 0% Phosphorylation Control (100% Inhibition Control)

The maximum Emission Ratio is established by the 0% Phosphorylation Control (100% Inhibition Control), which contains no ATP and therefore exhibits no kinase activity. This control yields 100% cleaved peptide in the Development Reaction.

### 100% Phosphorylation Control

The 100% Phosphorylation Control, which consists of a synthetically phosphorylated peptide of the same sequence as the peptide substrate, is designed to allow for the calculation of percent phosphorylation. This control yields a very low percentage of cleaved peptide in the Development Reaction.

The 0% Phosphorylation and 100% Phosphorylation Controls allow one to calculate the percent Phosphorylation achieved in a specific reaction well. Control wells do not include any kinase inhibitors.

### 0% Inhibition Control

The minimum Emission Ratio in a screen is established by the 0% Inhibition Control, which contains active kinase. This control is designed to produce a 10–50%\* phosphorylated peptide in the Kinase Reaction.

\* Cascade assays may produce up to 70% phosphorylated peptide.

### Known Inhibitor

A known inhibitor control standard curve, 10 point titration, is run for each individual kinase on the same plate as the kinase to ensure the kinase is inhibited within an expected IC<sub>50</sub> range previously determined.

The following controls are prepared for each concentration of Test Compound assayed:

### Development Reaction Interference

The Development Reaction Interference is established by comparing the Test Compound Control wells that do not contain ATP versus the 0% Phosphorylation Control (which does not contain the Test Compound). The expected value for a non-interfering compound should be 100%. Any value outside of 90% to 110% is flagged.

### Test Compound Fluorescence Interference

The Test Compound Fluorescence Interference is determined by comparing the Test Compound Control wells that do not contain the Kinase/Peptide Mixture (zero peptide control) versus the 0% Inhibition Control. The expected value for a non-fluorescence compound should be 0%. Any value > 20% is flagged.



## Z'-LYTE® Data Analysis

The following equations are used for each set of data points:

	Equation
<b>Correction for Background Fluorescence</b>	$FI_{\text{Sample}} - FI_{\text{TCFI Ctl}}$
<b>Emission Ratio</b> (using values corrected for background fluorescence)	$\frac{\text{Coumarin Emission (445 nm)}}{\text{Fluorescein Emission (520 nm)}}$
<b>% Phosphorylation (% Phos)</b>	$\left\{ 1 - \frac{(\text{Emission Ratio} \times F_{100\%}) - C_{100\%}}{(C_{0\%} - C_{100\%}) + [\text{Emission Ratio} \times (F_{100\%} - F_{0\%})]} \right\} * 100$
<b>% Inhibition</b>	$\left\{ 1 - \frac{\% \text{ Phos}_{\text{Sample}}}{\% \text{ Phos}_{0\% \text{ Inhibition Ctl}}} \right\} * 100$
<b>Z'</b> (using Emission Ratio values)	$1 - \frac{3 * \text{Stdev}_{0\% \text{ Phos Ctl}} + 3 * \text{Stdev}_{0\% \text{ Inhibition}}}{\text{Mean}_{0\% \text{ Phos Ctl}} - \text{Mean}_{0\% \text{ Inhibition}}}$
<b>Difference Between Data Points</b> (single point only)	$  \% \text{ Inhibition}_{\text{Point 1}} - \% \text{ Inhibition}_{\text{Point 2}}  $
<b>Development Reaction Interference (DRI)</b> (no ATP control)	$\frac{\text{Emission Ratio}_{\text{DRI Ctl}}}{\text{Emission Ratio}_{0\% \text{ Phos Ctl}}}$
<b>Test Compound Fluorescence Interference (TCFI)</b> (check both Coumarin and Fluorescein emissions)	$\frac{FI_{\text{TCFI Ctl}}}{FI_{0\% \text{ Inhibitor Ctl}}}$

FI = Fluorescence Intensity

C<sub>100%</sub> = Average Coumarin emission signal of the 100% Phos. Control

C<sub>0%</sub> = Average Coumarin emission signal of the 0% Phos. Control

F<sub>100%</sub> = Average Fluorescein emission signal of the 100% Phos. Control

F<sub>0%</sub> = Average Fluorescein emission signal of the 0% Phos. Control

DRI = Development Reaction Interference

TCFI = Test Compound Fluorescence Interference

### Graphing Software

SelectScreen® Kinase Profiling Service uses XLfit from IDBS. The dose response curve is curve fit to model number 205 (sigmoidal dose-response model). If the bottom of the curve does not fit between -20% & 20% inhibition, it is set to 0% inhibition. If the top of the curve does not fit between 70% and 130% inhibition, it is set to 100% inhibition.



## Kinase-Specific Assay Conditions - Direct Format

### **ABL1**

The 2X ABL1 / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.26 - 1.26 ng ABL1 and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

### **ABL1 E255K**

The 2X ABL1 E255K / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.75 - 5.18 ng ABL1 E255K and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

### **ABL1 G250E**

The 2X ABL1 G250E / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.07 - 7.92 ng ABL1 G250E and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

### **ABL1 T315I**

The 2X ABL1 T315I / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.36 - 8.18 ng ABL1 T315I and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

### **ABL1 Y253F**

The 2X ABL1 Y253F / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.73 - 5.24 ng ABL1 Y253F and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

### **ABL2 (Arg)**

The 2X ABL2 (Arg) / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.42 - 5.3 ng ABL2 (Arg) and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

### **ACVR1B (ALK4)**

The 2X ACVR1B (ALK4) / Ser/Thr 16 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 11.3 - 76.5 ng ACVR1B (ALK4) and 2 µM Ser/Thr 16 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

### **ADRBK1 (GRK2)**

The 2X ADRBK1 (GRK2) / Ser/Thr 16 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.86 - 67.6 ng ADRBK1 (GRK2) and 2 µM Ser/Thr 16 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

### **ADRBK2 (GRK3)**

The 2X ADRBK2 (GRK3) / Ser/Thr 16 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 9.93 - 39.8 ng ADRBK2 (GRK3) and 2 µM Ser/Thr 16 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

### **AKT1 (PKB alpha)**

The 2X AKT1 (PKB alpha) / Ser/Thr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.15 - 25 ng AKT1 (PKB alpha) and 2 µM Ser/Thr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

### **AKT2 (PKB beta)**

The 2X AKT2 (PKB beta) / Ser/Thr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1 - 40 ng AKT2 (PKB beta) and 2 µM Ser/Thr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

**AKT3 (PKB gamma)**

The 2X AKT3 (PKB gamma) / Ser/Thr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.62 - 8.3 ng AKT3 (PKB gamma) and 2 µM Ser/Thr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

**AMPK A1/B1/G1**

The 2X AMPK A1/B1/G1 / Ser/Thr 23 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.34 - 1.71 ng AMPK A1/B1/G1 and 2 µM Ser/Thr 23 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

**AMPK A2/B1/G1**

The 2X AMPK A2/B1/G1 / Ser/Thr 23 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 4.67 - 40 ng AMPK A2/B1/G1 and 2 µM Ser/Thr 23 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

**AURKA (Aurora A)**

The 2X AURKA (Aurora A) / Ser/Thr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.91 - 12 ng AURKA (Aurora A) and 2 µM Ser/Thr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**AURKB (Aurora B)**

The 2X AURKB (Aurora B) / Ser/Thr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.5 - 56 ng AURKB (Aurora B) and 2 µM Ser/Thr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**AURKC (Aurora C)**

The 2X AURKC (Aurora C) / Ser/Thr 19 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 9.57 - 133 ng AURKC (Aurora C) and 2 µM Ser/Thr 19 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent A is added.

**AXL**

The 2X AXL / Tyr 06 mixture is prepared in 50 mM HEPES pH 6.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 6.63 - 112 ng AXL and 2 µM Tyr 06 in 50 mM HEPES pH 7.0, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**BLK**

The 2X BLK / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.41 - 8 ng BLK and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**BMX**

The 2X BMX / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.38 - 45.9 ng BMX and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**BRSK1 (SAD1)**

The 2X BRSK1 (SAD1) / Ser/Thr 21 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.8 - 41.7 ng BRSK1 (SAD1) and 2 µM Ser/Thr 21 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**BTK**

The 2X BTK / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.04 - 21.2 ng BTK and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**CAMK1D (CaMKI delta)**

The 2X CAMK1D (CaMKI delta) / Ser/Thr 25 mixture is prepared in 50 mM HEPES pH 6.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 40 µg/ml Calmodulin. The final 10 µL Kinase Reaction consists of 11 - 133 ng CAMK1D (CaMKI delta) and 2 µM Ser/Thr 25 in 50 mM HEPES pH 7.0, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 20 µg/ml Calmodulin. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.





**CAMK2A (CaMKII alpha)**

The 2X CAMK2A (CaMKII alpha) / Ser/Thr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 40 µg/ml Calmodulin, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 0.38 - 3 ng CAMK2A (CaMKII alpha) and 2 µM Ser/Thr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 20 µg/ml Calmodulin, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

**CAMK2B (CaMKII beta)**

The 2X CAMK2B (CaMKII beta) / Ser/Thr 17 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 40 µg/ml Calmodulin, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 0.7 - 16.6 ng CAMK2B (CaMKII beta) and 2 µM Ser/Thr 17 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 20 µg/ml Calmodulin, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent A is added.

**CAMK2D (CaMKII delta)**

The 2X CAMK2D (CaMKII delta) / Ser/Thr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 40 µg/ml Calmodulin, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 0.11 - 2.16 ng CAMK2D (CaMKII delta) and 2 µM Ser/Thr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 20 µg/ml Calmodulin, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

**CAMK4 (CaMKIV)**

The 2X CAMK4 (CaMKIV) / Ser/Thr 13 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 40 µg/ml Calmodulin, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 4.92 - 90 ng CAMK4 (CaMKIV) and 2 µM Ser/Thr 13 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 20 µg/ml Calmodulin, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

**CDC42 BPA (MRCKA)**

The 2X CDC42 BPA (MRCKA) / Ser/Thr 13 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 10.2 - 54.2 ng CDC42 BPA (MRCKA) and 2 µM Ser/Thr 13 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

**CDC42 BPB (MRCKB)**

The 2X CDC42 BPB (MRCKB) / Ser/Thr 13 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.59 - 24 ng CDC42 BPB (MRCKB) and 2 µM Ser/Thr 13 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

**CDK1/cyclin B**

The 2X CDK1/cyclin B / Ser/Thr 18 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 5.65 - 46.4 ng CDK1/cyclin B and 2 µM Ser/Thr 18 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**CDK2/cyclin A**

The 2X CDK2/cyclin A / Ser/Thr 12 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.5 - 25 ng CDK2/cyclin A and 2 µM Ser/Thr 12 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

**CDK5/p25**

The 2X CDK5/p25 / Ser/Thr 12 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.18 - 1.15 ng CDK5/p25 and 2 µM Ser/Thr 12 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

**CDK5/p35**

The 2X CDK5/p35 / Ser/Thr 12 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.14 - 0.96 ng CDK5/p35 and 2 µM Ser/Thr 12 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

**CHEK1 (CHK1)**

The 2X CHEK1 (CHK1) / Ser/Thr 19 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.38 - 35 ng CHEK1 (CHK1) and 2 µM Ser/Thr 19 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent A is added.

**CHEK2 (CHK2)**

The 2X CHEK2 (CHK2) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.97 - 43.8 ng CHEK2 (CHK2) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**CLK1**

The 2X CLK1 / Ser/Thr 09 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 16.2 - 128 ng CLK1 and 2 µM Ser/Thr 09 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

**CLK2**

The 2X CLK2 / Ser/Thr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.97 - 11.6 ng CLK2 and 2 µM Ser/Thr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

**CLK3**

The 2X CLK3 / Ser/Thr 18 mixture is prepared in 50 mM Tris pH 8.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 10.4 - 45.6 ng CLK3 and 2 µM Ser/Thr 18 in 50 mM Tris / HEPES pH 8.0, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**CSF1R (FMS)**

The 2X CSF1R (FMS) / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.17 - 22 ng CSF1R (FMS) and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**CSK**

The 2X CSK / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.5 - 26 ng CSK and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**CSNK1A1 (CK1 alpha 1)**

The 2X CSNK1A1 (CK1 alpha 1) / Ser/Thr 11 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 2.62 - 20.7 ng CSNK1A1 (CK1 alpha 1) and 2 µM Ser/Thr 11 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

**CSNK1D (CK1 delta)**

The 2X CSNK1D (CK1 delta) / Ser/Thr 11 mixture is prepared in 50 mM Tris pH 8.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 17.5 - 104 ng CSNK1D (CK1 delta) and 2 µM Ser/Thr 11 in 50 mM Tris / HEPES pH 8.0, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

**CSNK1E (CK1 epsilon)**

The 2X CSNK1E (CK1 epsilon) / Ser/Thr 11 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.69 - 13.3 ng CSNK1E (CK1 epsilon) and 2 µM Ser/Thr 11 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

**CSNK1G1 (CK1 gamma 1)**

The 2X CSNK1G1 (CK1 gamma 1) / Ser/Thr 05 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.14 - 17.5 ng CSNK1G1 (CK1 gamma 1) and 2 µM Ser/Thr 05 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**CSNK1G2 (CK1 gamma 2)**

The 2X CSNK1G2 (CK1 gamma 2) / Ser/Thr 05 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.75 - 19.8 ng CSNK1G2 (CK1 gamma 2) and 2 µM Ser/Thr 05 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**CSNK1G3 (CK1 gamma 3)**

The 2X CSNK1G3 (CK1 gamma 3) / Ser/Thr 05 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.1 - 31.7 ng CSNK1G3 (CK1 gamma 3) and 2 µM Ser/Thr 05 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**CSNK2A1 (CK2 alpha 1)**

The 2X CSNK2A1 (CK2 alpha 1) / Ser/Thr 11 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.35 - 25.7 ng CSNK2A1 (CK2 alpha 1) and 2 µM Ser/Thr 11 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

**CSNK2A2 (CK2 alpha 2)**

The 2X CSNK2A2 (CK2 alpha 2) / Ser/Thr 11 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 5.75 - 31.5 ng CSNK2A2 (CK2 alpha 2) and 2 µM Ser/Thr 11 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

**DAPK3 (ZIPK)**

The 2X DAPK3 (ZIPK) / Ser/Thr 13 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 5.63 - 47.7 ng DAPK3 (ZIPK) and 2 µM Ser/Thr 13 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

**DCAMKL2 (DCK2)**

The 2X DCAMKL2 (DCK2) / Ser/Thr 17 mixture is prepared in 50 mM HEPES pH 6.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 12.6 - 57 ng DCAMKL2 (DCK2) and 2 µM Ser/Thr 17 in 50 mM HEPES pH 7.0, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent A is added.

**DNA-PK**

The 2X DNA-PK / Ser/Thr 26 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT, 5.0 µg/ml CT-DNA. The final 10 µL Kinase Reaction consists of 3.88 - 27.3 ng DNA-PK and 2 µM Ser/Thr 26 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT, 2.5 µg/ml CT-DNA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

**DYRK1A**

The 2X DYRK1A / Ser/Thr 18 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.68 - 12.5 ng DYRK1A and 2 µM Ser/Thr 18 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**DYRK1B**

The 2X DYRK1B / Ser/Thr 18 mixture is prepared in 50 mM HEPES pH 6.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 0.47 - 5.84 ng DYRK1B and 2 µM Ser/Thr 18 in 50 mM HEPES pH 7.0, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**DYRK3**

The 2X DYRK3 / Ser/Thr 09 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.92 - 10.7 ng DYRK3 and 2 µM Ser/Thr 09 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

**DYRK4**

The 2X DYRK4 / Ser/Thr 09 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 13 - 74.1 ng DYRK4 and 2 µM Ser/Thr 09 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

**EEF2K**

The 2X EEF2K / Ser/Thr 24 mixture is prepared in 50 mM HEPES pH 6.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 40 µg/ml Calmodulin. The final 10 µL Kinase Reaction consists of 5.53 - 60 ng EEF2K and 2 µM Ser/Thr 24 in 50 mM HEPES pH 7.0, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 20 µg/ml Calmodulin. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:4096 dilution of Development Reagent A is added.

**EGFR (ErbB1)**

The 2X EGFR (ErbB1) / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 1.57 - 7.5 ng EGFR (ErbB1) and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**EGFR (ErbB1) L858R**

The 2X EGFR (ErbB1) L858R / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 0.2 - 1.68 ng EGFR (ErbB1) L858R and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**EGFR (ErbB1) L861Q**

The 2X EGFR (ErbB1) L861Q / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 2.31 - 15.2 ng EGFR (ErbB1) L861Q and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**EGFR (ErbB1) T790M**

The 2X EGFR (ErbB1) T790M / Tyr 04 mixture is prepared in 50 mM HEPES pH 6.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.02% Na<sub>3</sub>N. The final 10 µL Kinase Reaction consists of 3.9 - 30.2 ng EGFR (ErbB1) T790M and 2 µM Tyr 04 in 50 mM HEPES pH 7.0, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.01% Na<sub>3</sub>N. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**EGFR (ErbB1) T790M L858R**

The 2X EGFR (ErbB1) T790M L858R / Tyr 04 mixture is prepared in 50 mM HEPES pH 6.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.02% Na<sub>3</sub>N. The final 10 µL Kinase Reaction consists of 0.38 - 4.22 ng EGFR (ErbB1) T790M L858R and 2 µM Tyr 04 in 50 mM HEPES pH 7.0, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.01% Na<sub>3</sub>N. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**EPHA1**

The 2X EPHA1 / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 5.52 - 80 ng EPHA1 and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**EPHA2**

The 2X EPHA2 / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.75 - 40 ng EPHA2 and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**EPHA4**

The 2X EPHA4 / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.45 - 13.8 ng EPHA4 and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**EPHA5**

The 2X EPHA5 / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.45 - 38.1 ng EPHA5 and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**EPHA8**

The 2X EPHA8 / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2 - 24.6 ng EPHA8 and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**EPHB1**

The 2X EPHB1 / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.39 - 39.4 ng EPHB1 and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**EPHB2**

The 2X EPHB2 / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.55 - 18 ng EPHB2 and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**EPHB3**

The 2X EPHB3 / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.78 - 43.8 ng EPHB3 and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**EPHB4**

The 2X EPHB4 / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.75 - 18 ng EPHB4 and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**ERBB2 (HER2)**

The 2X ERBB2 (HER2) / Tyr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT, 0.02% Na<sub>3</sub>N. The final 10 µL Kinase Reaction consists of 1.85 - 30.8 ng ERBB2 (HER2) and 2 µM Tyr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT, 0.01% Na<sub>3</sub>N. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**ERBB4 (HER4)**

The 2X ERBB4 (HER4) / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 2 - 23 ng ERBB4 (HER4) and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**FER**

The 2X FER / Tyr 05 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.59 - 13.9 ng FER and 2 µM Tyr 05 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**FES (FPS)**

The 2X FES (FPS) / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.65 - 5.21 ng FES (FPS) and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**FGFR1**

The 2X FGFR1 / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 0.41 - 3.5 ng FGFR1 and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**FGFR2**

The 2X FGFR2 / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 0.19 - 2.36 ng FGFR2 and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**FGFR3**

The 2X FGFR3 / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 2.25 - 20 ng FGFR3 and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**FGFR3 K650E**

The 2X FGFR3 K650E / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 0.6 - 4.72 ng FGFR3 K650E and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**FGFR4**

The 2X FGFR4 / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 2.44 - 105 ng FGFR4 and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**FGR**

The 2X FGR / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.75 - 13.5 ng FGR and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**FLT1 (VEGFR1)**

The 2X FLT1 (VEGFR1) / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 4.5 - 25 ng FLT1 (VEGFR1) and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**FLT3**

The 2X FLT3 / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.4 - 28 ng FLT3 and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**FLT3 D835Y**

The 2X FLT3 D835Y / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.06 - 1.48 ng FLT3 D835Y and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**FLT4 (VEGFR3)**

The 2X FLT4 (VEGFR3) / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 2 - 11.7 ng FLT4 (VEGFR3) and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**FRAP1 (mTOR)**

The 2X FRAP1 (mTOR) / Ser/Thr 11 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 10.5 - 44.8 ng FRAP1 (mTOR) and 2 µM Ser/Thr 11 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

**FRK (PTK5)**

The 2X FRK (PTK5) / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.1 - 51.9 ng FRK (PTK5) and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**FYN**

The 2X FYN / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 4.45 - 45.6 ng FYN and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**GRK4**

The 2X GRK4 / Ser/Thr 16 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.79 - 3.58 ng GRK4 and 2 µM Ser/Thr 16 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

**GRK5**

The 2X GRK5 / Ser/Thr 16 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 7.89 - 50 ng GRK5 and 2 µM Ser/Thr 16 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

**GRK6**

The 2X GRK6 / Ser/Thr 16 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 9.2 - 61.8 ng GRK6 and 2 µM Ser/Thr 16 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

**GRK7**

The 2X GRK7 / Ser/Thr 16 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 4 - 23 ng GRK7 and 2 µM Ser/Thr 16 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

**GSK3A (GSK3 alpha)**

The 2X GSK3A (GSK3 alpha) / Ser/Thr 09 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.1 - 0.6 ng GSK3A (GSK3 alpha) and 2 µM Ser/Thr 09 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

**GSK3B (GSK3 beta)**

The 2X GSK3B (GSK3 beta) / Ser/Thr 09 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.05 - 0.92 ng GSK3B (GSK3 beta) and 2 µM Ser/Thr 09 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

**HCK**

The 2X HCK / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.38 - 3.5 ng HCK and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**HIPK1 (Myak)**

The 2X HIPK1 (Myak) / Ser/Thr 09 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.69 - 24 ng HIPK1 (Myak) and 2 µM Ser/Thr 09 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

**HIPK2**

The 2X HIPK2 / Ser/Thr 09 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.53 - 46.7 ng HIPK2 and 2 µM Ser/Thr 09 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

**HIPK3 (YAK1)**

The 2X HIPK3 (YAK1) / Ser/Thr 18 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 30.8 - 136 ng HIPK3 (YAK1) and 2 µM Ser/Thr 18 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**HIPK4**

The 2X HIPK4 / Ser/Thr 18 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 10.9 - 88.9 ng HIPK4 and 2 µM Ser/Thr 18 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**IGF1R**

The 2X IGF1R / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.04 - 98.8 ng IGF1R and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**IKBKB (IKK beta)**

The 2X IKBKB (IKK beta) / Ser/Thr 05 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.93 - 8.02 ng IKBKB (IKK beta) and 2 µM Ser/Thr 05 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**IKBKE (IKK epsilon)**

The 2X IKBKE (IKK epsilon) / Ser/Thr 11 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.65 - 15.6 ng IKBKE (IKK epsilon) and 2 µM Ser/Thr 11 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

**INSR**

The 2X INSR / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 2.33 - 50 ng INSR and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**INSRR (IRR)**

The 2X INSRR (IRR) / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 2.19 - 22.2 ng INSRR (IRR) and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**IRAK4**

The 2X IRAK4 / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 3.45 - 63.6 ng IRAK4 and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**ITK**

The 2X ITK / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 4.69 - 144 ng ITK and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**JAK1**

The 2X JAK1 / Tyr 06 mixture is prepared in 50 mM HEPES pH 6.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 21.2 - 91.5 ng JAK1 and 2 µM Tyr 06 in 50 mM HEPES pH 7.0, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**JAK2**

The 2X JAK2 / Tyr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.06 - 0.81 ng JAK2 and 2 µM Tyr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**JAK2 JH1 JH2**

The 2X JAK2 JH1 JH2 / Tyr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.75 - 3.44 ng JAK2 JH1 JH2 and 2 µM Tyr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.



**JAK2 JH1 JH2 V617F**

The 2X JAK2 JH1 JH2 V617F / Tyr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 7.5 - 40.5 ng JAK2 JH1 JH2 V617F and 2 µM Tyr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**JAK3**

The 2X JAK3 / Tyr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.29 - 1.34 ng JAK3 and 2 µM Tyr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**KDR (VEGFR2)**

The 2X KDR (VEGFR2) / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.5 - 11.7 ng KDR (VEGFR2) and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**KIT**

The 2X KIT / Tyr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 1.7 - 15 ng KIT and 2 µM Tyr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**KIT T670I**

The 2X KIT T670I / Tyr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 4.34 - 75 ng KIT T670I and 2 µM Tyr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**LCK**

The 2X LCK / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.79 - 39.5 ng LCK and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**LTK (TYK1)**

The 2X LTK (TYK1) / Tyr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.66 - 55.5 ng LTK (TYK1) and 2 µM Tyr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**LYN A**

The 2X LYN A / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.36 - 4.07 ng LYN A and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**LYN B**

The 2X LYN B / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.89 - 14.8 ng LYN B and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**MAP3K9 (MLK1)**

The 2X MAP3K9 (MLK1) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 6.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 11.3 - 48 ng MAP3K9 (MLK1) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.0, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**MAP4K2 (GCK)**

The 2X MAP4K2 (GCK) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.25 - 3.5 ng MAP4K2 (GCK) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**MAP4K4 (HGK)**

The 2X MAP4K4 (HGK) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.23 - 2.5 ng MAP4K4 (HGK) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**MAP4K5 (KHS1)**

The 2X MAP4K5 (KHS1) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.62 - 6 ng MAP4K5 (KHS1) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**MAPK1 (ERK2)**

The 2X MAPK1 (ERK2) / Ser/Thr 03 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2 - 45.5 ng MAPK1 (ERK2) and 2 µM Ser/Thr 03 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**MAPK11 (p38 beta)**

The 2X MAPK11 (p38 beta) / Ser/Thr 15 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 10.6 - 95 ng MAPK11 (p38 beta) and 2 µM Ser/Thr 15 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**MAPK12 (p38 gamma)**

The 2X MAPK12 (p38 gamma) / Ser/Thr 03 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.47 - 4.8 ng MAPK12 (p38 gamma) and 2 µM Ser/Thr 03 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**MAPK13 (p38 delta)**

The 2X MAPK13 (p38 delta) / Ser/Thr 03 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.22 - 36.6 ng MAPK13 (p38 delta) and 2 µM Ser/Thr 03 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**MAPK14 (p38 alpha) Direct**

The 2X MAPK14 (p38 alpha) Direct / Ser/Thr 15 mixture is prepared in 50 mM HEPES pH 6.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 6.8 - 103 ng MAPK14 (p38 alpha) Direct and 2 µM Ser/Thr 15 in 50 mM HEPES pH 7.0, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**MAPK3 (ERK1)**

The 2X MAPK3 (ERK1) / Ser/Thr 03 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.97 - 37.4 ng MAPK3 (ERK1) and 2 µM Ser/Thr 03 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**MAPKAPK2**

The 2X MAPKAPK2 / Ser/Thr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.04 - 0.18 ng MAPKAPK2 and 2 µM Ser/Thr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

**MAPKAPK3**

The 2X MAPKAPK3 / Ser/Thr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.13 - 15.8 ng MAPKAPK3 and 2 µM Ser/Thr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

**MAPKAPK5 (PRAK)**

The 2X MAPKAPK5 (PRAK) / Ser/Thr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.08 - 18 ng MAPKAPK5 (PRAK) and 2 µM Ser/Thr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

**MARK1 (MARK)**

The 2X MARK1 (MARK) / Ser/Thr 21 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 14.9 - 240 ng MARK1 (MARK) and 2 µM Ser/Thr 21 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**MARK2**

The 2X MARK2 / Ser/Thr 21 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 37.2 - 186 ng MARK2 and 2 µM Ser/Thr 21 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**MARK3**

The 2X MARK3 / Ser/Thr 25 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.6 - 3 ng MARK3 and 2 µM Ser/Thr 25 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**MARK4**

The 2X MARK4 / Ser/Thr 25 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.5 - 2.14 ng MARK4 and 2 µM Ser/Thr 25 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**MATK (HYL)**

The 2X MATK (HYL) / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 8.5 - 117 ng MATK (HYL) and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**MELK**

The 2X MELK / Ser/Thr 17 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.08 - 6.48 ng MELK and 2 µM Ser/Thr 17 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent A is added.

**MERTK (cMER)**

The 2X MERTK (cMER) / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 0.68 - 7.8 ng MERTK (cMER) and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**MET (cMet)**

The 2X MET (cMet) / Tyr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.49 - 8 ng MET (cMet) and 2 µM Tyr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**MET M1250T**

The 2X MET M1250T / Tyr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3 - 27 ng MET M1250T and 2 µM Tyr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**MINK1**

The 2X MINK1 / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.9 - 20.8 ng MINK1 and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**MKNK1 (MNK1)**

The 2X MKNK1 (MNK1) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 13.5 - 54 ng MKNK1 (MNK1) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**MST1R (RON)**

The 2X MST1R (RON) / Tyr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.25 - 1.21 ng MST1R (RON) and 2 µM Tyr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**MST4**

The 2X MST4 / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 7.59 - 52.3 ng MST4 and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**MUSK**

The 2X MUSK / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 16.4 - 151 ng MUSK and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**MYLK2 (skMLCK)**

The 2X MYLK2 (skMLCK) / Ser/Thr 13 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 40 µg/ml Calmodulin, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 2.7 - 24.4 ng MYLK2 (skMLCK) and 2 µM Ser/Thr 13 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 20 µg/ml Calmodulin, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

**NEK1**

The 2X NEK1 / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.09 - 80 ng NEK1 and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**NEK2**

The 2X NEK2 / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.4 - 7.7 ng NEK2 and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**NEK4**

The 2X NEK4 / Ser/Thr 17 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 7.44 - 40.3 ng NEK4 and 2 µM Ser/Thr 17 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent A is added.

**NEK6**

The 2X NEK6 / Ser/Thr 22 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.79 - 6 ng NEK6 and 2 µM Ser/Thr 22 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:4096 dilution of Development Reagent A is added.

**NEK7**

The 2X NEK7 / Ser/Thr 22 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 7.5 - 103 ng NEK7 and 2 µM Ser/Thr 22 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:4096 dilution of Development Reagent A is added.

**NEK9**

The 2X NEK9 / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 1.8 - 22.4 ng NEK9 and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**NTRK1 (TRKA)**

The 2X NTRK1 (TRKA) / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 4.72 - 88.7 ng NTRK1 (TRKA) and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**NTRK2 (TRKB)**

The 2X NTRK2 (TRKB) / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 0.34 - 3.64 ng NTRK2 (TRKB) and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**NTRK3 (TRKC)**

The 2X NTRK3 (TRKC) / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.33 - 45.1 ng NTRK3 (TRKC) and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**PAK1**

The 2X PAK1 / Ser/Thr 19 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.75 - 30.8 ng PAK1 and 2 µM Ser/Thr 19 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent A is added.

**PAK2 (PAK65)**

The 2X PAK2 (PAK65) / Ser/Thr 20 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.29 - 6 ng PAK2 (PAK65) and 2 µM Ser/Thr 20 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

**PAK3**

The 2X PAK3 / Ser/Thr 20 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.13 - 22 ng PAK3 and 2 µM Ser/Thr 20 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

**PAK4**

The 2X PAK4 / Ser/Thr 20 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.1 - 0.75 ng PAK4 and 2 µM Ser/Thr 20 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

**PAK6**

The 2X PAK6 / Ser/Thr 20 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.69 - 5.22 ng PAK6 and 2 µM Ser/Thr 20 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

**PAK7 (KIAA1264)**

The 2X PAK7 (KIAA1264) / Ser/Thr 20 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.05 - 0.25 ng PAK7 (KIAA1264) and 2 µM Ser/Thr 20 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

**PASK**

The 2X PASK / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 5.5 - 22.5 ng PASK and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**PDGFRA (PDGFR alpha)**

The 2X PDGFRA (PDGFR alpha) / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 1.54 - 12.5 ng PDGFRA (PDGFR alpha) and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**PDGFRA D842V**

The 2X PDGFRA D842V / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 3.49 - 31.2 ng PDGFRA D842V and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**PDGFRA T674I**

The 2X PDGFRA T674I / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 16.3 - 65 ng PDGFRA T674I and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**PDGFRA V561D**

The 2X PDGFRA V561D / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 13.9 - 64.7 ng PDGFRA V561D and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**PDGFRB (PDGFR beta)**

The 2X PDGFRB (PDGFR beta) / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 8.75 - 50 ng PDGFRB (PDGFR beta) and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**PDK1 Direct**

The 2X PDK1 Direct / Ser/Thr 07 mixture is prepared in 50 mM Tris pH 8.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.02% Na<sub>3</sub>. The final 10 µL Kinase Reaction consists of 7.36 - 38.7 ng PDK1 Direct and 2 µM Ser/Thr 07 in 50 mM Tris / HEPES pH 8.0, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.01% Na<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**PHKG1**

The 2X PHKG1 / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 40 µg/ml Calmodulin, 0.02% Na<sub>3</sub>. The final 10 µL Kinase Reaction consists of 3.22 - 37.9 ng PHKG1 and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 20 µg/ml Calmodulin, 0.01% Na<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**PHKG2**

The 2X PHKG2 / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.3 - 10.4 ng PHKG2 and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**PIM1**

The 2X PIM1 / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.3 - 13.9 ng PIM1 and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**PIM2**

The 2X PIM2 / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.88 - 12.4 ng PIM2 and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**PKN1 (PRK1)**

The 2X PKN1 (PRK1) / Ser/Thr 07 mixture is prepared in 50 mM Tris pH 8.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.02% Na<sub>3</sub>. The final 10 µL Kinase Reaction consists of 0.75 - 60 ng PKN1 (PRK1) and 2 µM Ser/Thr 07 in 50 mM Tris / HEPES pH 8.0, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.01% Na<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**PLK1**

The 2X PLK1 / Ser/Thr 16 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.98 - 18 ng PLK1 and 2 µM Ser/Thr 16 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

**PLK2**

The 2X PLK2 / Ser/Thr 16 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 9.59 - 160 ng PLK2 and 2 µM Ser/Thr 16 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

**PLK3**

The 2X PLK3 / Ser/Thr 16 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.45 - 2.7 ng PLK3 and 2 µM Ser/Thr 16 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

**PRKACA (PKA)**

The 2X PRKACA (PKA) / Ser/Thr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.05 - 0.21 ng PRKACA (PKA) and 2 µM Ser/Thr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**PRKCA (PKC alpha)**

The 2X PRKCA (PKC alpha) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 2X Novel Lipid Mix, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 0.02 - 0.23 ng PRKCA (PKC alpha) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 1X Novel Lipid Mix, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**PRKCB1 (PKC beta I)**

The 2X PRKCB1 (PKC beta I) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 2X Novel Lipid Mix, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 0.03 - 0.48 ng PRKCB1 (PKC beta I) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 1X Novel Lipid Mix, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**PRKCB2 (PKC beta II)**

The 2X PRKCB2 (PKC beta II) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 2X Novel Lipid Mix, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 0.05 - 0.9 ng PRKCB2 (PKC beta II) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 1X Novel Lipid Mix, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**PRKCD (PKC delta)**

The 2X PRKCD (PKC delta) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 2X Novel Lipid Mix, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 0.2 - 1 ng PRKCD (PKC delta) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 1X Novel Lipid Mix, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**PRKCE (PKC epsilon)**

The 2X PRKCE (PKC epsilon) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 2X Novel Lipid Mix, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 0.14 - 2.7 ng PRKCE (PKC epsilon) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 1X Novel Lipid Mix, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**PRKCG (PKC gamma)**

The 2X PRKCG (PKC gamma) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 2X Novel Lipid Mix, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 0.03 - 0.2 ng PRKCG (PKC gamma) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 1X Novel Lipid Mix, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**PRKCH (PKC eta)**

The 2X PRKCH (PKC eta) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 2X Novel Lipid Mix, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 0.14 - 1.5 ng PRKCH (PKC eta) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 1X Novel Lipid Mix, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**PRKCI (PKC iota)**

The 2X PRKCI (PKC iota) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 2X Novel Lipid Mix, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 0.75 - 6 ng PRKCI (PKC iota) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 1X Novel Lipid Mix, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**PRKCN (PKD3)**

The 2X PRKCN (PKD3) / Ser/Thr 17 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.25 - 16.7 ng PRKCN (PKD3) and 2 µM Ser/Thr 17 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent A is added.

**PRKCQ (PKC theta)**

The 2X PRKCQ (PKC theta) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 2X Novel Lipid Mix, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 0.04 - 0.36 ng PRKCQ (PKC theta) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 1X Novel Lipid Mix, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**PRKCZ (PKC zeta)**

The 2X PRKCZ (PKC zeta) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM CaCl<sub>2</sub>, 2X Novel Lipid Mix, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 1.58 - 8.25 ng PRKCZ (PKC zeta) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 500 µM EGTA, 2 mM CaCl<sub>2</sub>, 1X Novel Lipid Mix, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**PRKD1 (PKC mu)**

The 2X PRKD1 (PKC mu) / Ser/Thr 17 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.56 - 8.5 ng PRKD1 (PKC mu) and 2 µM Ser/Thr 17 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent A is added.

**PRKD2 (PKD2)**

The 2X PRKD2 (PKD2) / Ser/Thr 17 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.64 - 8.34 ng PRKD2 (PKD2) and 2 µM Ser/Thr 17 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent A is added.

**PRKG1**

The 2X PRKG1 / Ser/Thr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 20 µM cGMP. The final 10 µL Kinase Reaction consists of 0.25 - 1.55 ng PRKG1 and 2 µM Ser/Thr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 10 µM cGMP. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**PRKG2 (PKG2)**

The 2X PRKG2 (PKG2) / Ser/Thr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 20 µM cGMP. The final 10 µL Kinase Reaction consists of 0.008 - 0.7 ng PRKG2 (PKG2) and 2 µM Ser/Thr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 10 µM cGMP. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**PRKX**

The 2X PRKX / Ser/Thr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.38 - 10 ng PRKX and 2 µM Ser/Thr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**PTK2 (FAK)**

The 2X PTK2 (FAK) / Tyr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 32.5 - 207 ng PTK2 (FAK) and 2 µM Tyr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

**PTK2B (FAK2)**

The 2X PTK2B (FAK2) / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 7.52 - 45.9 ng PTK2B (FAK2) and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**PTK6 (Brk)**

The 2X PTK6 (Brk) / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 11.8 - 65 ng PTK6 (Brk) and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.





**RET**

The 2X RET / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.49 - 4.06 ng RET and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**RET V804L**

The 2X RET V804L / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.52 - 5.02 ng RET V804L and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**RET Y791F**

The 2X RET Y791F / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.86 - 5.3 ng RET Y791F and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**ROCK1**

The 2X ROCK1 / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1 - 8 ng ROCK1 and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**ROCK2**

The 2X ROCK2 / Ser/Thr 13 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.64 - 4.88 ng ROCK2 and 2 µM Ser/Thr 13 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

**ROS1**

The 2X ROS1 / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.75 - 20.8 ng ROS1 and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**RPS6KA1 (RSK1)**

The 2X RPS6KA1 (RSK1) / Ser/Thr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 4.89 - 45.2 ng RPS6KA1 (RSK1) and 2 µM Ser/Thr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

**RPS6KA2 (RSK3)**

The 2X RPS6KA2 (RSK3) / Ser/Thr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.27 - 15.6 ng RPS6KA2 (RSK3) and 2 µM Ser/Thr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

**RPS6KA3 (RSK2)**

The 2X RPS6KA3 (RSK2) / Ser/Thr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.24 - 1.36 ng RPS6KA3 (RSK2) and 2 µM Ser/Thr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

**RPS6KA4 (MSK2)**

The 2X RPS6KA4 (MSK2) / Ser/Thr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 12.4 - 200 ng RPS6KA4 (MSK2) and 2 µM Ser/Thr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**RPS6KA5 (MSK1)**

The 2X RPS6KA5 (MSK1) / Ser/Thr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.04 - 22.7 ng RPS6KA5 (MSK1) and 2 µM Ser/Thr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**RPS6KA6 (RSK4)**

The 2X RPS6KA6 (RSK4) / Ser/Thr 20 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.19 - 2.5 ng RPS6KA6 (RSK4) and 2 µM Ser/Thr 20 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

**RPS6KB1 (p70S6K)**

The 2X RPS6KB1 (p70S6K) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.87 - 20 ng RPS6KB1 (p70S6K) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**SGK (SGK1)**

The 2X SGK (SGK1) / Ser/Thr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.09 - 1 ng SGK (SGK1) and 2 µM Ser/Thr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

**SGK2**

The 2X SGK2 / Ser/Thr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.21 - 3 ng SGK2 and 2 µM Ser/Thr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

**SGKL (SGK3)**

The 2X SGKL (SGK3) / Ser/Thr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.09 - 0.74 ng SGKL (SGK3) and 2 µM Ser/Thr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

**SNF1LK2**

The 2X SNF1LK2 / Ser/Thr 25 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.62 - 9.94 ng SNF1LK2 and 2 µM Ser/Thr 25 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**SRC**

The 2X SRC / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.56 - 36 ng SRC and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**SRC N1**

The 2X SRC N1 / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1 - 15.5 ng SRC N1 and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**SRMS (Srm)**

The 2X SRMS (Srm) / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.76 - 18.3 ng SRMS (Srm) and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent B is added.

**SRPK1**

The 2X SRPK1 / Ser/Thr 18 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.75 - 21.8 ng SRPK1 and 2 µM Ser/Thr 18 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**SRPK2**

The 2X SRPK2 / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.88 - 60 ng SRPK2 and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**STK22B (TSSK2)**

The 2X STK22B (TSSK2) / Ser/Thr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 5.55 - 37.5 ng STK22B (TSSK2) and 2 µM Ser/Thr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

**STK22D (TSSK1)**

The 2X STK22D (TSSK1) / Ser/Thr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.45 - 11.4 ng STK22D (TSSK1) and 2 µM Ser/Thr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

**STK23 (MSSK1)**

The 2X STK23 (MSSK1) / Ser/Thr 18 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 11.3 - 65 ng STK23 (MSSK1) and 2 µM Ser/Thr 18 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**STK24 (MST3)**

The 2X STK24 (MST3) / Ser/Thr 07 mixture is prepared in 50 mM Tris pH 8.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 14.7 - 70 ng STK24 (MST3) and 2 µM Ser/Thr 07 in 50 mM Tris / HEPES pH 8.0, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**STK25 (YSK1)**

The 2X STK25 (YSK1) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 3.07 - 75 ng STK25 (YSK1) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**STK3 (MST2)**

The 2X STK3 (MST2) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 6.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 7.5 - 38.7 ng STK3 (MST2) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.0, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**STK4 (MST1)**

The 2X STK4 (MST1) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 6.25 - 160 ng STK4 (MST1) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**SYK**

The 2X SYK / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.77 - 9.55 ng SYK and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**TAOK2 (TAO1)**

The 2X TAOK2 (TAO1) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 4.14 - 34 ng TAOK2 (TAO1) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:32768 dilution of Development Reagent A is added.

**TBK1**

The 2X TBK1 / Ser/Thr 05 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.7 - 9.72 ng TBK1 and 2 µM Ser/Thr 05 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

**TEK (Tie2)**

The 2X TEK (Tie2) / Tyr 05 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 4 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 1 - 9 ng TEK (Tie2) and 2 µM Tyr 05 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

**TXK**

The 2X TXK / Tyr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.29 - 93.6 ng TXK and 2 µM Tyr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**TYK2**

The 2X TYK2 / Tyr 03 mixture is prepared in 50 mM HEPES pH 6.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 15.9 - 106 ng TYK2 and 2 µM Tyr 03 in 50 mM HEPES pH 7.0, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.



**TYRO3 (RSE)**

The 2X TYRO3 (RSE) / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.15 - 25.8 ng TYRO3 (RSE) and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**YES1**

The 2X YES1 / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.41 - 18 ng YES1 and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent A is added.

**ZAP70**

The 2X ZAP70 / Tyr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT, 0.02% NaN<sub>3</sub>. The final 10 µL Kinase Reaction consists of 18.2 - 165 ng ZAP70 and 2 µM Tyr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT, 0.01% NaN<sub>3</sub>. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.



## ***Kinase-Specific Assay Conditions - Cascade Format***

### **BRAF**

The 2X BRAF / inactive MAP2K1 (MEK1) / inactive MAPK1 (ERK2)/Ser/Thr 03 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.03 - 0.12 ng BRAF, 1X inactive MAP2K1 (MEK1) / inactive MAPK1 (ERK2), and 2 µM Ser/Thr 03 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

### **BRAF V599E**

The 2X BRAF V599E / inactive MAP2K1 (MEK1) / inactive MAPK1 (ERK2)/Ser/Thr 03 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.001 - 0.003 ng BRAF V599E, 1X inactive MAP2K1 (MEK1) / inactive MAPK1 (ERK2), and 2 µM Ser/Thr 03 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

### **MAP2K1 (MEK1)**

The 2X MAP2K1 (MEK1) / inactive MAPK1 (ERK2)/Ser/Thr 03 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.1 - 0.4 ng MAP2K1 (MEK1), 105 ng inactive MAPK1 (ERK2), and 2 µM Ser/Thr 03 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

### **MAP2K2 (MEK2)**

The 2X MAP2K2 (MEK2) / inactive MAPK1 (ERK2)/Ser/Thr 03 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.13 - 4.5 ng MAP2K2 (MEK2), 105 ng inactive MAPK1 (ERK2), and 2 µM Ser/Thr 03 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

### **MAP2K6 (MKK6)**

The 2X MAP2K6 (MKK6) / inactive MAPK12 (p38 gamma)/Ser/Thr 03 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 18.8 - 75 ng MAP2K6 (MKK6), 100 ng inactive MAPK12 (p38 gamma), and 2 µM Ser/Thr 03 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

### **MAP3K8 (COT)**

The 2X MAP3K8 (COT) / inactive MAP2K1 (MEK1) / inactive MAPK1 (ERK2)/Ser/Thr 03 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.88 - 3.5 ng MAP3K8 (COT), 1X inactive MAP2K1 (MEK1) / inactive MAPK1 (ERK2), and 2 µM Ser/Thr 03 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

### **MAPK10 (JNK3)**

The 2X MAPK10 (JNK3) / inactive MAPKAPK2/Ser/Thr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 0.29 - 1.17 ng MAPK10 (JNK3), 100 ng inactive MAPKAPK2, and 2 µM Ser/Thr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

### **MAPK14 (p38 alpha)**

The 2X MAPK14 (p38 alpha) / inactive MAPKAPK2/Ser/Thr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.003 - 0.01 ng MAPK14 (p38 alpha), 5 ng inactive MAPKAPK2, and 2 µM Ser/Thr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

### **MAPK8 (JNK1)**

The 2X MAPK8 (JNK1) / inactive MAPKAPK2/Ser/Thr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 2 - 8 ng MAPK8 (JNK1), 25 ng inactive MAPKAPK2, and 2 µM Ser/Thr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.



**MAPK9 (JNK2)**

The 2X MAPK9 (JNK2) / inactive MAPKAPK2/Ser/Thr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 0.48 - 1.92 ng MAPK9 (JNK2), 100 ng inactive MAPKAPK2, and 2 µM Ser/Thr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

**PDK1**

The 2X PDK1 / inactive Akt2 (PKB beta)/Ser/Thr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.5 - 10 ng PDK1, 150 ng inactive Akt2 (PKB beta), and 2 µM Ser/Thr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:2048 dilution of Development Reagent A is added.

**RAF1 (cRAF) Y340D Y341D**

The 2X RAF1 (cRAF) Y340D Y341D / inactive MAP2K1 (MEK1) / inactive MAPK1 (ERK2)/Ser/Thr 03 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.002 - 0.006 ng RAF1 (cRAF) Y340D Y341D, 1X inactive MAP2K1 (MEK1) / inactive MAPK1 (ERK2), and 2 µM Ser/Thr 03 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl<sub>2</sub>, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

### Table of Kinase ATP Km Bins and Inhibitor Validation

The table below provides specifications and data around each kinase. The representative IC50 value with a known inhibitor for each kinase was determined at the ATP bin nearest to the ATP Km app, unless indicated with an asterisk (\*) in which case the IC50 value was determined at 100 µM ATP.

Kinase	Z'-LYTE Substrate	ATP Km app (µM)	ATP Bin (µM)	Inhibitor	IC50 (nM)
ABL1	Tyr 02	12	10	Tyrphostin AG1478	434
ABL1 E255K	Tyr 02	4.2	5	Staurosporine	104
ABL1 G250E	Tyr 02	5.4	5	Staurosporine	53.0
ABL1 T315I	Tyr 02	2.8	5	Staurosporine	18.4
ABL1 Y253F	Tyr 02	11.8	10	Staurosporine	79.0
ABL2 (Arg)	Tyr 02	30	25	Tyrphostin AG1478	1050
ACVR1B (ALK4)	Ser/Thr 16	1.6	5	SB 431542	792
ADRBK1 (GRK2)	Ser/Thr 16	13.3	10	Staurosporine	2240
ADRBK2 (GRK3)	Ser/Thr 16	11.7	10	Staurosporine	2360
AKT1 (PKB alpha)	Ser/Thr 06	75	75	Staurosporine	36.9
AKT2 (PKB beta)	Ser/Thr 06	200	200	Staurosporine	117
AKT3 (PKB gamma)	Ser/Thr 06	100	100	Staurosporine	25.5
AMPK A1/B1/G1	Ser/Thr 23	43	50	Staurosporine	0.680
AMPK A2/B1/G1	Ser/Thr 23	148	150	Staurosporine	1.50
AURKA (Aurora A)	Ser/Thr 01	10	10	Staurosporine	3.92
AURKB (Aurora B)	Ser/Thr 01	81	75	Staurosporine	11.3
AURKC (Aurora C)	Ser/Thr 19	26	25	Staurosporine	8.72
AXL	Tyr 06	43	50	Staurosporine	11.6
BLK	Tyr 01	30	25	Tyrphostin AG1478	1940
BMX	Tyr 01	107	100	Staurosporine	63.0
BRAF	Ser/Thr 03	Cascade	100	Staurosporine	80.0 *
BRAF V599E	Ser/Thr 03	Cascade	100	Staurosporine	104 *
BRSK1 (SAD1)	Ser/Thr 21	32	25	Staurosporine	2.44
BTK	Tyr 01	36	25	Tyrphostin AG1478	5950
CAMK1D (CaMKI delta)	Ser/Thr 25	28	25	Staurosporine	37.6
CAMK2A (CaMKII alpha)	Ser/Thr 04	10	10	Staurosporine	1.80
CAMK2B (CaMKII beta)	Ser/Thr 17	76	75	Staurosporine	2.07
CAMK2D (CaMKII delta)	Ser/Thr 04	6	5	Staurosporine	0.693
CAMK4 (CaMKIV)	Ser/Thr 13	18	25	Staurosporine	247
CDC42 BPA (MRCKA)	Ser/Thr 13	1	5	Staurosporine	31.3
CDC42 BPB (MRCKB)	Ser/Thr 13	1	5	Staurosporine	15.0
CDK1/cyclin B	Ser/Thr 18	34	25	Staurosporine	10.3
CDK2/cyclin A	Ser/Thr 12	31	25	Staurosporine	5.88
CDK5/p25	Ser/Thr 12	17	10	Staurosporine	9.70
CDK5/p35	Ser/Thr 12	8	10	Staurosporine	9.16
CHEK1 (CHK1)	Ser/Thr 19	53	50	Staurosporine	4.49
CHEK2 (CHK2)	Ser/Thr 07	84	75	Staurosporine	26.2
CLK1	Ser/Thr 09	24	25	Staurosporine	143
CLK2	Ser/Thr 06	30	25	Staurosporine	18.8
CLK3	Ser/Thr 18	128	150	Staurosporine	2340
CSF1R (FMS)	Tyr 01	450	500	Tyrphostin AG1478	6020
CSK	Tyr 02	15	10	Staurosporine	97.3
CSNK1A1 (CK1 alpha 1)	Ser/Thr 11	2	5	TBB	17600
CSNK1D (CK1 delta)	Ser/Thr 11	4	5	TBB	3680
CSNK1E (CK1 epsilon)	Ser/Thr 11	2	5	TBB	4830



Z'-LYTE® Screening Protocol and Assay Conditions

Revised 15-Dec-2010

Page 32 of 35

Kinase	Z'-LYTE Substrate	ATP Km app (µM)	ATP Bin (µM)	Inhibitor	IC50 (nM)
CSNK1G1 (CK1 gamma 1)	Ser/Thr 05	2	5	Chetomin	109
CSNK1G2 (CK1 gamma 2)	Ser/Thr 05	5	5	TBB	26500
CSNK1G3 (CK1 gamma 3)	Ser/Thr 05	4.4	5	TBB	69900
CSNK2A1 (CK2 alpha 1)	Ser/Thr 11	4	5	TBB	2980
CSNK2A2 (CK2 alpha 2)	Ser/Thr 11	46	50	TBB	2370
DAPK3 (ZIPK)	Ser/Thr 13	2.5	5	Staurosporine	45.4
DCAMKL2 (DCK2)	Ser/Thr 17	149.5	150	Staurosporine	77.8
DNA-PK	Ser/Thr 26	17	25	PI-103	12.2
DYRK1A	Ser/Thr 18	99.7	100	Staurosporine	32.0
DYRK1B	Ser/Thr 18	86	75	Staurosporine	10.0
DYRK3	Ser/Thr 09	4.7	5	Staurosporine	176
DYRK4	Ser/Thr 09	2.2	5	TBB	12900
EEF2K	Ser/Thr 24	15	10	NH125	18700
EGFR (ErbB1)	Tyr 04	11.5	10	Staurosporine	149
EGFR (ErbB1) L858R	Tyr 04	44.2	50	Staurosporine	151
EGFR (ErbB1) L861Q	Tyr 04	12.7	10	Staurosporine	87.9
EGFR (ErbB1) T790M	Tyr 04	10	10	Staurosporine	2.90
EGFR (ErbB1) T790M L858R	Tyr 04	26	25	Staurosporine	0.600
EPHA1	Tyr 02	18.5	25	Staurosporine	83.3
EPHA2	Tyr 01	65.4	75	Staurosporine	290
EPHA4	Tyr 02	106	100	Staurosporine	104
EPHA5	Tyr 01	129	150	Staurosporine	114
EPHA8	Tyr 02	123	100	Staurosporine	182
EPHB1	Tyr 02	61.4	50	Staurosporine	172
EPHB2	Tyr 02	66	75	Staurosporine	216
EPHB3	Tyr 02	70	75	PP2	763
EPHB4	Tyr 01	115	100	Tyrphostin AG1478	1590
ERBB2 (HER2)	Tyr 06	14.1	10	Tyrphostin AG1478	211
ERBB4 (HER4)	Tyr 01	5	5	Tyrphostin AG1478	143
FER	Tyr 05	18.5	25	Staurosporine	4.79
FES (FPS)	Tyr 01	30	25	Staurosporine	4.58
FGFR1	Tyr 04	20	25	Staurosporine	18.3
FGFR2	Tyr 04	1	5	Staurosporine	5.33
FGFR3	Tyr 04	80	75	Staurosporine	67.4
FGFR3 K650E	Tyr 04	6	5	Staurosporine	8.50
FGFR4	Tyr 04	162	150	Staurosporine	274
FGR	Tyr 02	10	10	Tyrphostin AG1478	919
FLT1 (VEGFR1)	Tyr 04	158	150	Staurosporine	36.9
FLT3	Tyr 02	470	500	Tyrphostin AG1478	2940
FLT3 D835Y	Tyr 02	18.8	25	Staurosporine	0.478
FLT4 (VEGFR3)	Tyr 04	3.8	5	Staurosporine	5.82
FRAP1 (mTOR)	Ser/Thr 11	11	10	PI-103	31.0
FRK (PTK5)	Tyr 01	51.7	50	Staurosporine	17.1
FYN	Tyr 02	85	75	Tyrphostin AG1478	14500
GRK4	Ser/Thr 16	12	10	Staurosporine	173
GRK5	Ser/Thr 16	3	5	Staurosporine	260
GRK6	Ser/Thr 16	11.7	10	Staurosporine	119
GRK7	Ser/Thr 16	10.5	10	Staurosporine	8.23
GSK3A (GSK3 alpha)	Ser/Thr 09	9	10	Staurosporine	9.17
GSK3B (GSK3 beta)	Ser/Thr 09	7	10	Staurosporine	17.4
HCK	Tyr 02	24	25	Tyrphostin AG1478	550
HIPK1 (Myak)	Ser/Thr 09	5	5	TBB	4470
HIPK2	Ser/Thr 09	17	10	Staurosporine	1720
HIPK3 (YAK1)	Ser/Thr 18	22	25	TBB	5104





Z'-LYTE® Screening Protocol and Assay Conditions

Revised 15-Dec-2010

Page 33 of 35

Kinase	Z'-LYTE Substrate	ATP Km app (µM)	ATP Bin (µM)	Inhibitor	IC50 (nM)
HIPK4	Ser/Thr 18	39	50	Staurosporine	798
IGF1R	Tyr 01	140	150	Staurosporine	228
IKBKB (IKK beta)	Ser/Thr 05	5	5	Staurosporine	451
IKBKE (IKK epsilon)	Ser/Thr 11	16	10	Staurosporine	8.50
INSR	Tyr 01	20	25	Staurosporine	215
INSRR (IRR)	Tyr 04	55	50	Staurosporine	295
IRAK4	Ser/Thr 07	34	25	Staurosporine	32.6
ITK	Tyr 01	5.6	5	Staurosporine	48.6
JAK1	Tyr 06	87	75	Staurosporine	4.20
JAK2	Tyr 06	31	25	Staurosporine	1.00
JAK2 JH1 JH2	Tyr 06	46	50	Staurosporine	1.40
JAK2 JH1 JH2 V617F	Tyr 06	49	50	Staurosporine	1.90
JAK3	Tyr 06	14	10	Staurosporine	2.50
KDR (VEGFR2)	Tyr 01	78	75	Staurosporine	7.45
KIT	Tyr 06	284	300	Staurosporine	492
KIT T670I	Tyr 06	220	200	Staurosporine	197
LCK	Tyr 02	45	50	Tyrphostin AG1478	1510
LTK (TYK1)	Tyr 06	82	75	Staurosporine	17.0
LYN A	Tyr 02	25	25	Tyrphostin AG1478	208
LYN B	Tyr 02	25	25	Tyrphostin AG1478	244
MAP2K1 (MEK1)	Ser/Thr 03	Cascade	100	Staurosporine	14.7 *
MAP2K2 (MEK2)	Ser/Thr 03	Cascade	100	Staurosporine	15.2 *
MAP2K6 (MKK6)	Ser/Thr 03	Cascade	100	Staurosporine	10.5 *
MAP3K8 (COT)	Ser/Thr 03	Cascade	100	Staurosporine	81.6 *
MAP3K9 (MLK1)	Ser/Thr 07	73	75	Staurosporine	5.80
MAP4K2 (GCK)	Ser/Thr 07	109	100	Staurosporine	1.70
MAP4K4 (HGK)	Ser/Thr 07	12.7	10	Staurosporine	2.56
MAP4K5 (KHS1)	Ser/Thr 07	55	50	Staurosporine	2.34
MAPK1 (ERK2)	Ser/Thr 03	100	100	Staurosporine	2470
MAPK10 (JNK3)	Ser/Thr 04	Cascade	100	Staurosporine	2520 *
MAPK11 (p38 beta)	Ser/Thr 15	39	50	PP2	3530
MAPK12 (p38 gamma)	Ser/Thr 03	16	10	Staurosporine	373
MAPK13 (p38 delta)	Ser/Thr 03	13	10	Staurosporine	405
MAPK14 (p38 alpha)	Ser/Thr 04	Cascade	100	Staurosporine	6410 *
MAPK14 (p38 alpha) Direct	Ser/Thr 15	497	500	PP2	3030
MAPK3 (ERK1)	Ser/Thr 03	45	50	Staurosporine	3290
MAPK8 (JNK1)	Ser/Thr 04	Cascade	100	Staurosporine	2310 *
MAPK9 (JNK2)	Ser/Thr 04	Cascade	100	Staurosporine	3300 *
MAPKAPK2	Ser/Thr 04	2.5	5	Staurosporine	717
MAPKAPK3	Ser/Thr 04	200	200	Staurosporine	45900
MAPKAPK5 (PRAK)	Ser/Thr 04	14	10	Staurosporine	871
MARK1 (MARK)	Ser/Thr 21	7	5	Staurosporine	18.6
MARK2	Ser/Thr 21	12	10	Staurosporine	45.3
MARK3	Ser/Thr 25	7	5	Staurosporine	2.46
MARK4	Ser/Thr 25	16	10	Staurosporine	2.08
MATK (HYL)	Tyr 01	350	300	Staurosporine	1020
MELK	Ser/Thr 17	30	25	Staurosporine	3.50
MERTK (cMER)	Tyr 02	15	10	Staurosporine	42.5
MET (cMet)	Tyr 06	64	50	Staurosporine	163
MET M1250T	Tyr 06	14.1	10	Staurosporine	308
MINK1	Ser/Thr 07	26.5	25	Staurosporine	2.32
MKNK1 (MNK1)	Ser/Thr 07	103	100	Staurosporine	132
MST1R (RON)	Tyr 06	10.7	10	Staurosporine	73.9
MST4	Ser/Thr 07	28	25	Staurosporine	3.96



<b>Kinase</b>	<b>Z'-LYTE Substrate</b>	<b>ATP Km app (µM)</b>	<b>ATP Bin (µM)</b>	<b>Inhibitor</b>	<b>IC50 (nM)</b>
MUSK	Tyr 04	49.5	50	Staurosporine	9.30
MYLK2 (skMLCK)	Ser/Thr 13	310	300	Staurosporine	236
NEK1	Ser/Thr 07	118.7	100	Staurosporine	122
NEK2	Ser/Thr 07	150	150	Staurosporine	5880
NEK4	Ser/Thr 17	56.2	50	Staurosporine	879
NEK6	Ser/Thr 22	15	10	Chetomin	59.0
NEK7	Ser/Thr 22	62	50	Chetomin	274
NEK9	Ser/Thr 07	90	100	Staurosporine	2200
NTRK1 (TRKA)	Tyr 01	425	400	Staurosporine	3.23
NTRK2 (TRKB)	Tyr 01	22	25	Staurosporine	0.916
NTRK3 (TRKC)	Tyr 01	54	50	Staurosporine	2.54
PAK1	Ser/Thr 19	48.5	50	Staurosporine	3.00
PAK2 (PAK65)	Ser/Thr 20	89	75	Staurosporine	30.0
PAK3	Ser/Thr 20	101	100	Staurosporine	15.3
PAK4	Ser/Thr 20	3	5	Staurosporine	9.71
PAK6	Ser/Thr 20	8	10	Staurosporine	4.59
PAK7 (KIAA1264)	Ser/Thr 20	4	5	Staurosporine	5.05
PASK	Ser/Thr 07	53.3	50	Staurosporine	96.0
PDGFRA (PDGFR alpha)	Tyr 04	9	10	Staurosporine	8.36
PDGFRA D842V	Tyr 04	5.3	5	Staurosporine	14.3
PDGFRA T674I	Tyr 04	93	100	Staurosporine	8.12
PDGFRA V561D	Tyr 04	48	50	Staurosporine	1.90
PDGFRB (PDGFR beta)	Tyr 04	100	100	Staurosporine	6.44
PDK1	Ser/Thr 06	Cascade	100	Staurosporine	12.5 *
PDK1 Direct	Ser/Thr 07	27	25	Staurosporine	51.0
PHKG1	Ser/Thr 07	63.3	75	Staurosporine	1.15
PHKG2	Ser/Thr 07	10	10	Staurosporine	3.35
PIM1	Ser/Thr 07	407	400	TBB	1970
PIM2	Ser/Thr 07	3	5	Staurosporine	64.5
PKN1 (PRK1)	Ser/Thr 07	40	50	Staurosporine	1.60
PLK1	Ser/Thr 16	12.8	10	Staurosporine	823
PLK2	Ser/Thr 16	29.6	25	Staurosporine	634
PLK3	Ser/Thr 16	47.8	50	TBB	3630
PRKACA (PKA)	Ser/Thr 01	4	5	Staurosporine	3.36
PRKCA (PKC alpha)	Ser/Thr 07	37	25	Staurosporine	2.24
PRKCB1 (PKC beta I)	Ser/Thr 07	250	200	Staurosporine	3.59
PRKCB2 (PKC beta II)	Ser/Thr 07	225	200	Staurosporine	2.18
PRKCD (PKC delta)	Ser/Thr 07	30	25	Staurosporine	1.83
PRKCE (PKC epsilon)	Ser/Thr 07	35	25	Staurosporine	2.42
PRKCG (PKC gamma)	Ser/Thr 07	25	25	Staurosporine	1.85
PRKCH (PKC eta)	Ser/Thr 07	35	25	Staurosporine	5.08
PRKCI (PKC iota)	Ser/Thr 07	25	25	Staurosporine	406
PRKCN (PKD3)	Ser/Thr 17	26	25	Staurosporine	1.95
PRKCQ (PKC theta)	Ser/Thr 07	100	100	Staurosporine	4.13
PRKCZ (PKC zeta)	Ser/Thr 07	4	5	Staurosporine	1110
PRKD1 (PKC mu)	Ser/Thr 17	15	10	Staurosporine	5.92
PRKD2 (PKD2)	Ser/Thr 17	27	25	Staurosporine	4.97
PRKG1	Ser/Thr 01	20	25	Staurosporine	5.39
PRKG2 (PKG2)	Ser/Thr 01	177	150	Staurosporine	1.76
PRKX	Ser/Thr 01	17	10	Staurosporine	3.89
PTK2 (FAK)	Tyr 07	46	50	Staurosporine	66.3
PTK2B (FAK2)	Tyr 01	5	5	Staurosporine	34.1
PTK6 (Brk)	Tyr 01	82	75	Staurosporine	1250
RAF1 (cRAF) Y340D Y341D	Ser/Thr 03	Cascade	100	Staurosporine	55.4 *



Kinase	Z'-LYTE Substrate	ATP Km app (µM)	ATP Bin (µM)	Inhibitor	IC50 (nM)
RET	Tyr 02	11	10	Staurosporine	5.07
RET V804L	Tyr 02	5	5	Staurosporine	5.90
RET Y791F	Tyr 02	12	10	Staurosporine	4.40
ROCK1	Ser/Thr 07	3.1	5	Staurosporine	7.18
ROCK2	Ser/Thr 13	39.5	50	Staurosporine	5.36
ROS1	Tyr 01	61.4	50	Staurosporine	5.44
RPS6KA1 (RSK1)	Ser/Thr 06	6.5	5	Staurosporine	1.57
RPS6KA2 (RSK3)	Ser/Thr 06	10.7	10	Staurosporine	1.06
RPS6KA3 (RSK2)	Ser/Thr 06	18	10	Staurosporine	0.865
RPS6KA4 (MSK2)	Ser/Thr 01	14.1	10	Staurosporine	3.93
RPS6KA5 (MSK1)	Ser/Thr 01	39	50	Staurosporine	5.44
RPS6KA6 (RSK4)	Ser/Thr 20	30	25	Staurosporine	0.599
RPS6KB1 (p70S6K)	Ser/Thr 07	17	10	Staurosporine	6.92
SGK (SGK1)	Ser/Thr 06	36	25	Staurosporine	19.8
SGK2	Ser/Thr 06	50	50	Staurosporine	85.1
SGKL (SGK3)	Ser/Thr 06	12	10	Staurosporine	89.0
SNF1LK2	Ser/Thr 25	59	50	Staurosporine	1.22
SRC	Tyr 02	50	50	Tyrphostin AG1478	3160
SRC N1	Tyr 02	50	50	Tyrphostin AG1478	1980
SRMS (Srm)	Tyr 01	126.9	150	Staurosporine	323
SRPK1	Ser/Thr 18	32	25	Staurosporine	560
SRPK2	Ser/Thr 07	17.6	25	Staurosporine	2200
STK22B (TSSK2)	Ser/Thr 04	3	5	Staurosporine	20.3
STK22D (TSSK1)	Ser/Thr 04	9	10	Staurosporine	0.918
STK23 (MSSK1)	Ser/Thr 18	69	75	Staurosporine	3530
STK24 (MST3)	Ser/Thr 07	56	50	Staurosporine	10.4
STK25 (YSK1)	Ser/Thr 07	83	75	Staurosporine	7.24
STK3 (MST2)	Ser/Thr 07	50	50	Staurosporine	6.51
STK4 (MST1)	Ser/Thr 07	48.9	50	Staurosporine	11.2
SYK	Tyr 02	24.8	25	Staurosporine	0.708
TAOK2 (TAO1)	Ser/Thr 07	322	300	Staurosporine	36.3
TBK1	Ser/Thr 05	31	25	Staurosporine	1.73
TEK (Tie2)	Tyr 05	16.7	10	Staurosporine	120
TXK	Tyr 06	96	100	Staurosporine	35.0
TYK2	Tyr 03	23	25	Staurosporine	1.80
TYRO3 (RSE)	Tyr 02	27.7	25	Staurosporine	11.1
YES1	Tyr 02	155	150	Tyrphostin AG1478	2620
ZAP70	Tyr 07	2	5	Staurosporine	432