Z′-LYTE™ KINASE ASSAY KIT – SER/THR 9 PEPTIDE PROTOCOL

Invitrogen™ discovery screening

Part # PV3324

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1.0 INTRODUCTION

Protein kinases and phosphatases regulate many critical biological mechanisms, including metabolism and cell growth, proliferation, and differentiation. Aberrations in the activity of the kinases and phosphatases involved in signal transduction have been linked to many human diseases. The discovery of more than 600 kinases and phosphatases encoded by the human genome has spurred development of rapid screening techniques for potential drugs against these enzymes.

We have developed the proprietary Z′-LYTE™ technology to address the need for quickly available assays to identify potential kinase and phosphatase inhibitors. This robust, room-temperature, homogeneous assay method uses fluorescence resonance energy transfer (FRET) between coumarin and fluorescein for detection. Reaction progress is quantitated with a ratiometric approach that reduces the effects of well-to-well variations. Therefore the results produce both low coefficients of variation (CVs) and high Z′-factor values, even when only a small percentage of the substrate is phosphorylated. Z′-LYTE™ technology is highly compatible with automated high-throughput screening (HTS) systems and can readily meet the growing demand for new assays to screen for inhibitors of a broad array of tyrosine and serine/threonine protein kinases and phosphatases.

The Z′-LYTE™ Kinase Assay Kit–Ser/Thr 9 Peptide is designed to accurately and reliably screen potential kinase inhibitors in a 20-µL, two-hour, room-temperature reaction. The reagent volumes listed in the Components Section are sufficient for 800 (20-µL) assays in 384-well assay plates; however, the assay is readily modified for ultra-miniaturized HTS applications (to as small as a 1.6-µL assay volume) with no loss of quality. This Z′-LYTE™ Kinase Assay Kit provides a flexible, addition-only, screening assay that yields Z′-factor values >0.7. For a list of kinases identified that phosphorylate the Z′-LYTE™ Ser/Thr 9 Peptide, refer to **www.invitrogen.com/zlyte**.

2.0 ASSAY THEORY

The Z′-LYTE™ biochemical assay employs a FRET-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 Reaction), the Kinase transfers the γ-phosphate of ATP to a single serine or threonine residue in the synthetic peptide substrate. In the secondary reaction (the Development Reaction), a site-specific protease (the Development Reagent) recognizes and cleaves non-phosphorylated peptides. Phosphorylated peptides exhibit suppressed cleavage by the Development Reagent. Cleavage disrupts FRET between the donor (*i.e.*, coumarin) and acceptor (*i.e.*, fluorescein) fluorophores on the peptide, whereas uncleaved, phosphorylated 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, quantitates reaction progress, as shown in the equation below.

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

This ratiometric method for quantitating reaction progress offers the significant benefit of reducing data fluctuations arising from well-to-well variations in peptide concentration and signal intensities. As a result, the assay generates data with very high Z′-factor values (>0.7), even when a low percentage of the peptide substrate is phosphorylated.

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).

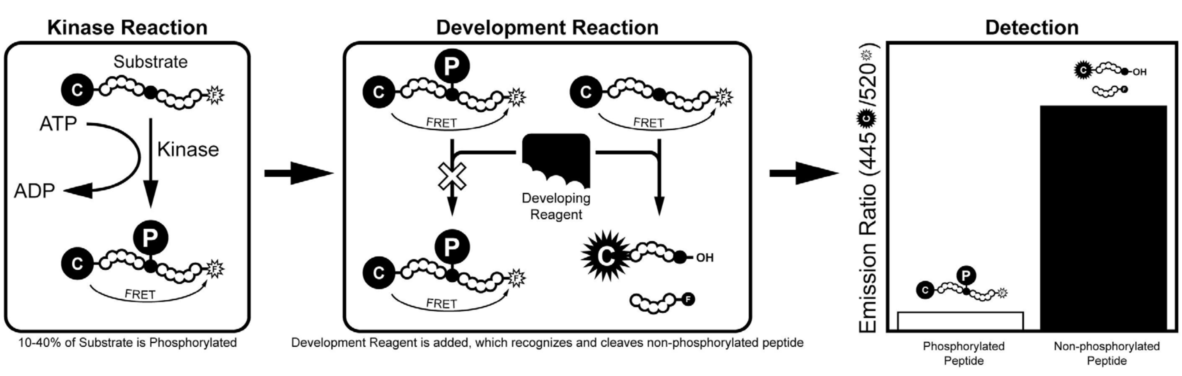


Figure 1. Schematic diagram of the Z′-LYTE™ biochemical assay.

3.0 Z′-LYTE™ KINASE ASSAY KIT–SER/THR 9 PEPTIDE MATERIALS SUPPLIED

Reagent	Description	Quantity	Part No.
Z′-LYTE™ Ser/Thr 9 Peptide*	1 mM in DMSO	20 µL	PV3325
Z′-LYTE™ Ser/Thr 9 Phospho-peptide*	1 mM in DMSO	10 µL	PV3326
5X Kinase Buffer	250 mM HEPES (pH 7.5), 50 mM $MgCl_2$, 5 mM EGTA, 0.05% BRIJ-35	4 mL	PV3189
ATP	10 mM in water	500 µL	PV3227
Development Reagent A	Proprietary Reagent	400 µL	PV3297
Development Buffer B	Proprietary Buffer	20 mL	P3127
Stop Reagent	Proprietary Reagent	5 mL	P3094

* Both the Ser/Thr 9 Peptide and the Ser/Thr 9 Phospho-peptide contain a priming phosphate at the +4 position to direct the kinase.

4.0 MATERIALS REQUIRED, BUT NOT SUPPLIED

- Kinase
- A fluorescence plate reader with the appropriate filter sets installed for detecting the fluorescence emission signals of coumarin and fluorescein. The recommended excitation wavelength is 400 nm and the recommended emission wavelengths are 445 nm and 520 nm, respectively. Select an excitation filter with a bandwidth appropriate for coumarin but not fluorescein. Select emission filter sets with appropriate bandwidths so that the emission signals of coumarin and fluorescein do not overlap. We chose 400 nm (12 nm bandwidth) for the excitation wavelengths and 445 nm (12 nm bandwidth) and 520 nm (12 nm bandwidth) for the emission wavelengths. Other similar filter sets may be suitable.
- 384-well assay plates. We recommend Corning® 384-well low-volume assay plates for serial dilutions (Corning® Part No. 3676). Other low-volume plates, while not tested, may be suitable.
- 12-channel, multi-channel pipette and any pipetting devices capable of accurately delivering repeated volumes of 2.5 µL and 5 µL.
- 96-well assay plate that can accommodate 300 µL.

5.0 STORAGE AND STABILITY

The Z′-LYTE™ Kinase Assay Kit is shipped on dry ice. Store entire kit at -80°C. Following initial use, store as detailed in the Table below. All reagents are stable for 6 months from the date of purchase, if stored and handled properly.

Reagent	Part No.	Storage Temperature	Notes
Z′-LYTE™ Ser/Thr 9 Peptide	PV3325	-20°C	
Z′-LYTE™ Ser/Thr 9 Phospho-peptide	PV3326	-20°C	
5X Kinase Buffer	PV3189	20–30°C	
ATP	PV3227	-20°C	
Development Reagent A	PV3297	-80°C	Avoid more than 5 freeze/thaw cycles
Development Buffer B	P3127	20–30°C	
Stop Reagent	P3094	-80°C	Avoid more than 5 freeze/thaw cycles

6.0 Z′-LYTE™ ASSAY CONSIDERATIONS

This kit contains sufficient reagents to perform 800 (20-µL) assays in 384-well plates. This flexible assay can accommodate alternative reaction conditions, such as changes in assay volumes, kinase and Development Reagent A concentrations, reaction times, and incubation temperatures.

6.1 Assay Controls

The 0% Phosphorylation and 100% Phosphorylation Controls allow you to calculate the percent phosphorylation achieved in a specific reaction well. The 0% Inhibition and 0% Phosphorylation (100% Inhibition) Controls define the dynamic range in a screen. Control wells do not include any kinase inhibitors.

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 will yield 100% cleaved peptide in the Development Reaction.

100% Phosphorylation Control

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

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 recommended 20–40% phosphorylated peptide in the Kinase Reaction and to yield 60–80% cleaved peptide in the Development Reaction.

6.2 Stability of Reagent Dilutions and Assay Signal

All diluted reagents are stable at room temperature for 6 hours, and with no loss of assay performance. Once developed, the Emission Ratio of the 20-µL assay does not change appreciably for up to 18 hours, if the assay plate is covered and protected from light.

6.3 Solvent Tolerances

The 10-µL Kinase Reaction can tolerate up to 2% DMSO without affecting assay results.

6.4 Assay Plates

We recommend Corning® 384-well low volume non-binding surface assay plates with a working volume range of 2–35 µL. Use comparable black, low binding assay plates when using larger or smaller assay volumes.

6.5 Assay Volumes

The final assay volume is 20 µL. You can successfully adapt the protocol for different reaction volumes (1.6–100 µL) if you use the component concentrations specified in this protocol.

6.6 Incubation Temperature

For optimal results, perform the assay at 20–25°C. If temperatures fall below this range, increase the incubation times for the Kinase and Development Reactions.

6.7 Kinase Reaction

Determine the optimal kinase concentration, ATP concentration, and reaction time empirically for each kinase (**Section 7.0**). We recommend using near-K_M ATP concentrations and a kinase concentration that phosphorylates 20–40% of the Z′-LYTE™ Ser/Thr 9 Peptide in a one-hour, room-temperature incubation.

6.8 Development Reaction

At the concentration specified in this protocol, Development Reagent A will completely cleave all non-phosphorylated Z′-LYTE™ Ser/Thr 9 Peptides in the one-hour, room-temperature Development Reaction.

7.0 ASSAY OPTIMIZATION PROCEDURE

This section provides a recommended method for optimizing the Z′-LYTE™ Kinase Assay Kit–Ser/Thr 9 Peptide for use with a particular kinase. The only variable is the kinase concentration. You can easily modify the protocol to examine the effects of ATP concentration or incubation time on the assay. Use the resulting experimental data as a guide for choosing an appropriate kinase concentration within the linear range to obtain the desired percent phosphorylation with an acceptable Z′-factor value. Experimental factors, such as incubation time and reaction temperature, affect the actual percent phosphorylation obtained. The volumes provided below are sufficient for 400 (20-µL) assays.

7.1 Reagent Preparation

Note: Thaw and store the kinase and Development Reagent A on ice prior to preparation of dilutions. Equilibrate all other assay components to room temperature.

1X Kinase Buffer

Dilute 2 mL 5X Kinase Buffer to 1X with water and any supplements required by the kinase.

2X Kinase Solution

Determine the desired maximum final kinase concentration for the assay. Prepare 300 µL of kinase in 1X Kinase Buffer to 2X the desired maximum concentration. Mix gently by pipetting; **do not vortex**.

Peptide/ATP Mixture

Determine the desired ATP concentration for the assay. Prepare 2250 µL of Peptide/ATP Mixture by diluting 9 µL Z′-LYTE™ Ser/Thr 9 Peptide and ATP in 1X Kinase Buffer. Use enough ATP so that the final concentration of ATP in this mixture is 2X the desired final concentration used in the assay. Mix thoroughly.

Phospho-peptide Solution

Add 2 µL Z′-LYTE™ Ser/Thr 9 Phospho-peptide to 498 µL 1X Kinase Buffer.

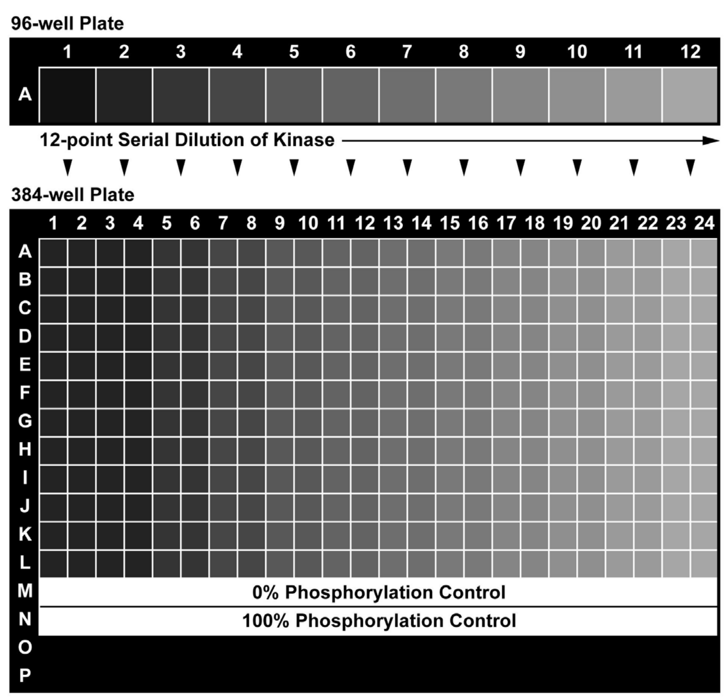
Development Solution

Combine 58 µL Development Reagent A with 2142 µL Development Buffer B to make the Development Solution. Mix gently by pipetting; **do not vortex**.

7.2 Kinase Dilution

Note: Use a 96-well plate that can accommodate 300 µL.

1. Dispense 140 µL 1X Kinase Buffer to wells A2–A12 of a 96-well plate. Do not add buffer to well A1, because it will contain the highest kinase concentration.
2. Dispense 280 µL Kinase Solution to well A1 of the 96-well plate.
3. Perform a two-fold serial dilution of the Kinase Solution by titering 140 µL from well A1 through well A12. Discard the final 140 µL so that 140 µL is left in wells A1-A12 of the 96-well plate.
4. Using a 12-channel pipette, transfer 5 µL diluted kinase from each well in row A (wells A1–A12) of the 96-well plate to duplicate columns of the first 12 rows (rows A – L) of a 384-well assay plate, as shown in the template to the right.



7.3 Kinase Reaction (384-well plate)

1. Dispense 5 µL 1X Kinase Buffer to each well in rows M and N (wells M1–N24).
2. Dispense 5 µL Phospho-peptide Solution to each well in row N.
3. Add 5 µL Peptide/ATP Mixture to each well in rows A–M.
4. Shake the assay plate on a plate shaker for 30 seconds to mix the reactions thoroughly.
5. Incubate the assay plate for one hour at room temperature (20–25°C).

7.4 Development Reaction

1. Add 5 µL Development Solution to each well in rows A–N.
2. Shake the assay plate on a plate shaker for 30 seconds to mix the reactions thoroughly.
3. Incubate the assay plate for one hour at room temperature (20–25°C).

7.5 Stop Step and Fluorescence Detection

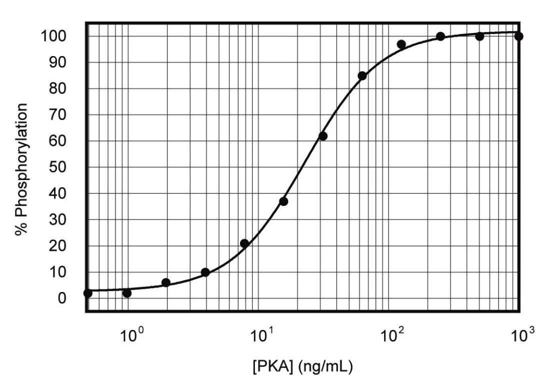
1. Add 5 µL Stop Reagent to each well in rows A–N.
2. Shake the assay plate on a plate shaker for 30 seconds to insure homogenous reaction mixtures.
3. Measure the Coumarin (Ex. 400 nm, Em. 445 nm) and Fluorescein (Ex. 400 nm, Em. 520 nm) emission signals on a fluorescence plate reader.

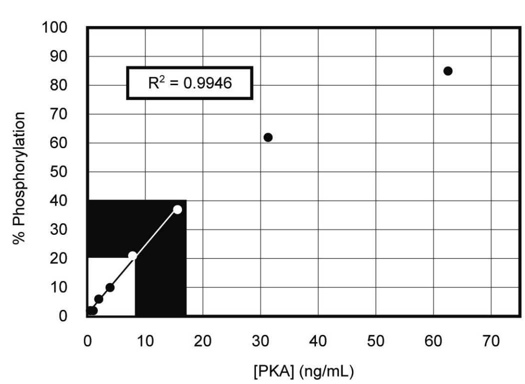
7.6 Data Analysis

Refer to **Section 9.0** for instructions on calculating the Emission Ratio, Percent Phosphorylation, and Z′-factor value for each kinase concentration tested following the Assay Optimization Procedure. Choose the appropriate kinase concentration for the Screening Procedure (**Section 8.0**) based upon the Z′-factor values within the linear range. We recommend using near-K_M ATP concentrations and a Kinase concentration that phosphorylates 20–40% of the Z′-LYTE™ Ser/Thr 9 Peptide in a one-hour, room-temperature incubation.

Table 1. Percent Phosphorylation and Z′-factor values corresponding to Kinase concentration. Representative sample data generated for PKA (Part No. P2912) with the Z′-LYTE™ Kinase Assay Kit–Ser/Thr 1 Peptide

[PKA] (ng/mL) in Kinase Reaction	Percent Phosphorylation	Z′-factor Value
0.49	2%	< 0.50
0.98	2%	< 0.50
1.95	6%	0.53
3.91	10%	0.67
7.81	21%	0.86
15.6	37%	0.87
31.3	62%	0.94
62.5	85%	0.96
125	97%	0.97
250	100%	0.97
500	100%	0.97
1000	100%	0.97





8.0 SCREENING PROCEDURE

Determine the necessary assay parameters, such as reaction times; incubation temperatures; and Kinase and ATP concentrations, to produce the desired extent of phosphorylation in the Kinase Reaction before performing the screening procedure. Use this protocol as a guideline for performing a primary screen for kinase inhibitors and for characterizing hits to determine their potencies. The volumes provided below are sufficient for 400 (20-µL) assays.

8.1 Reagent Preparation

Note: Thaw and store the kinase and Development Reagent A on ice prior to preparation of dilutions. Equilibrate all other assay components to room temperature.

1.33X Kinase Buffer

Dilute 2 mL of 5X Kinase Buffer to 1.33X with water and any supplements required by the kinase. In the screen, because the test compounds are in 4% DMSO, the 10-µL Kinase Reaction will contain all the kinase components in 1X Kinase Buffer and 1% DMSO.

4X Test Compounds

Prepare single concentrations (for primary screens) or serial dilutions (for characterizing hits) of the test compounds in 4% DMSO (in water) at four times the concentrations desired in the 10-µL Kinase Reactions.

Kinase/Peptide Mixture

Prepare 2250 µL of a Kinase/Peptide Mixture by diluting the kinase to 2X the empirically determined optimal concentration (See **Section 7.0**) and the Z′-LYTE™ Ser/Thr 9 Peptide to 4 µM (9 µL) in 1.33X Kinase Buffer. Mix gently by pipetting; **do not vortex**.

Phospho-peptide Solution

Add 2 µL of Z′-LYTE™ Ser/Thr 9 Phospho-peptide to 498 µL of 1.33X Kinase Buffer. Mix thoroughly.

ATP Solution

Prepare 1110 µL of an ATP Solution by diluting the 10-mM ATP in 1.33X Kinase Buffer to 4X the desired ATP concentration.

Development Solution

Combine 58 µL Development Reagent A with 2142 µL Development Buffer B to make the Development Solution. Mix gently by pipetting; **do not vortex**.

8.2 Assay Protocol

Table 2. Protocol for the Z′-LYTE™ Kinase Assay Kit. Add each component in the following order at the appropriate time points. In Step 1, initiate the Kinase Reaction by adding ATP.

		Assay Reaction(s)	Controls		
	Reagents	**Kinase + Test Compound**	**100% Inhibition (no ATP)**	**0% Inhibition (with ATP)**	**100% Phosphorylation**
Step 1	**Kinase Reaction (Primary Reaction)**				
	4X Test Compound (4% DMSO)	2.5 µL			
	4% DMSO		2.5 µL	2.5 µL	2.5 µL
	Kinase/Peptide Mixture	5 µL	5 µL	5 µL	
	Phospho-peptide Solution				5 µL
	1.33 X Kinase Buffer		2.5 µL		2.5 µL
	4X ATP Solution	2.5 µL		2.5 µL	

Mix assay plate and incubate the 10-µL Kinase Reaction for 1 hour at room temperature. ↓ **The Kinase Reaction contains 1X inhibitor, 1X Kinase, 1X ATP and 2 µM Z′-LYTE™ Ser/Thr 9 Peptide**

Step 2	**Development Reaction (Secondary Reaction)**				
	Development Solution	5 µL	5 µL	5 µL	5 µL

Mix assay plate and incubate the 15-µL Development Reaction for 1 hour at room temperature. ↓

Step 3	**Stop Step and Fluorescence Detection**				
	Stop Reagent	5 µL	5 µL	5 µL	5 µL

↓

Mix assay plate and measure fluorescence signals.
The 20-µL (final volume) assay contains 1 µM Z′-LYTE™ Ser/Thr 9 Peptide.

8.3 Sample Data

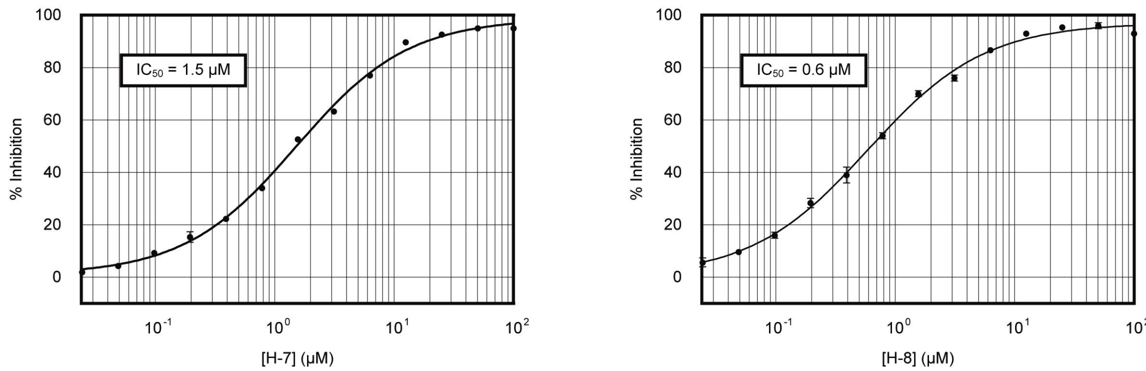


Figure 2. Dose-dependent inhibition of PKA by H-7 and H-8 in the Z′-LYTE™ Kinase Assay Kit. Two inhibitors, H-7 and H-8, were titrated in the Z′-LYTE™ Kinase Assay Kit – Ser/Thr 1 Peptide (Part No. PV3174) to determine their potencies against PKA. Both inhibitors were serially diluted two-fold across 20 wells. The 10 µL Kinase Reactions included 2 µM Ser/Thr 1 Peptide, 10 ng/mL PKA (Part No. P2912 Lot No. 27744B), and 4 µM ATP. The experimentally derived IC_{50} values for Staurosporine and Genistein, defined as the inhibitor concentration that produces a half-maximal value, were 1.5 and 0.6 µM, respectively. Error bars represent one standard deviation from the mean of three replicates. These assays were performed in a Corning® 384-well low volume assay plate and data were generated on a TECAN Safire™ monochromator-based fluorescence plate reader [Ex400; Em445; Em520. All filters had a 12-nm bandwidth.]. Curve fitting and data presentation were performed using Prism® software from GraphPad Software, Inc.

	MSK1	PKA	ROCK-II	AKT	PKCα
Literature Value	120 nM	135 nM	270 nM	2.60 µM	31.7 µM
Z′-LYTE™	140 nM	220 nM	890 nM	3.60 µM	77.0 µM

Table 3. Selectivity Profiling. The table above shows the results of Z′-LYTE™ IC_{50} determinations for the PKA inhibitor H-89 with several other kinases, and compares these data to the corresponding literature values. These data demonstrate that Z′-LYTE™ assays generate IC_{50} values that are very comparable to the literature values and provide a similar rank order of compound potency (PKA and MSK1 used Z′-LYTE™ Ser/Thr 1 Peptide; ROCK-II and PKCα used Z′-LYTE™ Ser/Thr 7 Peptide; Akt used Z′-LYTE™ Ser/Thr 6 Peptide; ATP concentration for MSK1, PKA, ROCK-II, and AKT was 100 µM; and ATP concentration for PKCα was 10 µM).

9.0 DATA ANALYSIS

9.1 Calculate Emission Ratio

The Emission Ratio for each well on the assay plate is calculated by dividing the coumarin emission signal (445 nm) by the fluorescein emission signal (520 nM).

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

9.2 Calculate Percent Phosphorylation

The extent of phosphorylation of each sample well (containing kinase) is determined according to the 0% and 100% Phosphorylation Control wells. There is a non-linear relationship between Emission Ratio and Phosphorylation **(see Figure 3)**, which the following equation accounts for:

$$\% \text{ Phosphorylation} = 1 - \frac{(\text{Emission Ratio} \times F_{100\%}) - C_{100\%}}{(C_{0\%} - C_{100\%}) + [\text{Emission Ratio} \times (F_{100\%} - F_{0\%})]}$$

where:

Emission Ratio = Coumarin/Fluorescein ratio of sample wells

$C_{100\%}$ = Average Coumarin emission signal of the 100% Phos. Control

$C_{0\%}$ = Average Coumarin emission signal of the 0% Phos. Control

$F_{100\%}$ = Average Fluorescein emission signal of the 100% Phos. Control

$F_{0\%}$ = Average Fluorescein emission signal of the 0% Phos. Control

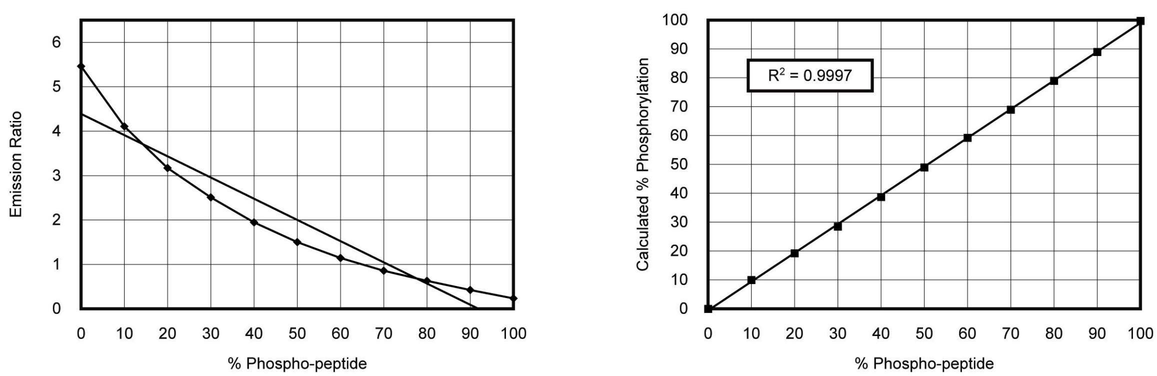


Figure 3. In a 384-well plate, various concentrations of Z′-LYTE™ Ser/Thr 9 Phospho-peptide were mixed with Z′-LYTE™ Ser/Thr 9 Peptide, each to a final concentration of 2 µM total Peptide in 10 µL of Kinase Buffer. The plate was incubated for one-hour at room-temperature with the Development Solution to cleave all non-phosphorylated peptide. Coumarin and Fluorescein emission signals were measured on a Tecan Safire™. The Emission Ratio and the Percent Phosphorylation of each sample were calculated and plotted against the Percent of Phospho-peptide. The degree of linearity of Emission Ratio to Percent Phospho-peptide is peptide-dependent. Peptides that demonstrate a larger change in magnitude of the Fluorescein signals between the 0% and 100% Phosphorylation Controls will demonstrate a greater degree of non-linearity between Emission Ratio and Percent Phospho-peptide, necessitating linearizing the emission ratios using the equation.

9.3 Calculate Z′-factor Values

The Z′-factor indicates the quality of an assay; Z′-factor values of 0.5 or greater classify an assay as excellent. The Assay Optimization Procedure (see **Section 7.0**) uses the wells in row O, which contain no kinase, as the 100% Inhibition Control wells, and each well in the rows containing kinase as the 0% Inhibition Control wells.

1. Calculate the Emission Ratio for each well.
2. Calculate the average and standard deviations of the ratios in each row
3. Calculate the Z′-factor value, using the following equation:

$$Z'\text{-Factor} = 1 - \frac{(3 \times \sigma_{100\%\ \text{Inhibition}}) + (3 \times \sigma_{0\%\ \text{Inhibition}})}{\mu_{100\%\ \text{Inhibition}} - \mu_{0\%\ \text{Inhibition}}}$$

10.0 REFERENCES

1. Rodems, S. *et al.* (2002) *ASSAY Drug Devel. Technol.* **1:**9-19.
2. Kleman-Leyer, K. *et al.* (2003) *Drug Disc. Devel.* **6:**81-2.
3. Frame, S. and Cohen, P. (2001) *Biocehm J.* **359:**1-16.
4. Zhang, J.H. *et al.* (1999) *J. Biomol. Screen.* **4:**67-73.
5. Davies, S.P. *et al.* (2000) *Biochem. J.* **351:**95-105.
6. Chijiwa, T. *et al.* (1990) *J. Biol. Chem.* **265:**5267-72.

11.0 PURCHASER NOTIFICATION

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