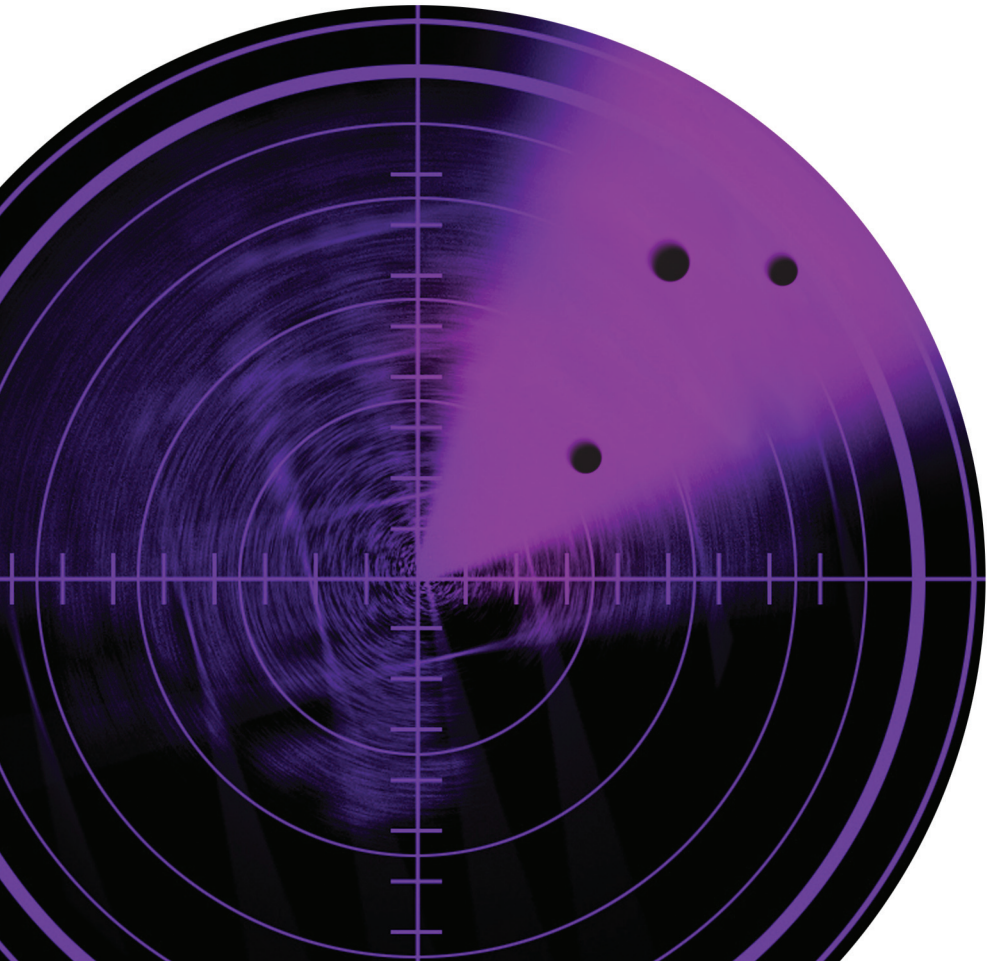
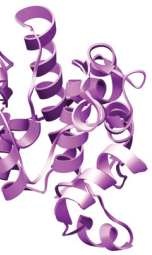
Drug Discovery

Your target is our aim

Kinase biochemical assays



invitrogen™



Boost your kinase research with the most comprehensive set of proteins and assay technologies

- → Access our collection of over 300 high-quality, ready-to-use purified human kinases
- → Choose from four robust fluorescent biochemical assay formats, each optimized for high-throughput screening
- → Enhance your research with our other kinase tools like cellular assays and profiling services

The discovery of reversible protein phosphorylation by Fischer and Krebs in 1954 has led to the understanding that protein kinases play a central role in the regulation of most cellular processes. Given the importance of the coordinated action of these enzymes, it is not surprising that amplification, overexpression, and misregulation of protein kinases is a cause or consequence of many diseases.

Protein kinases represent the largest enzyme family encoded by the human genome, with 518 members. All kinases share a highly conserved catalytic domain spanning 250–300 amino acids. The catalytic domain is composed of 12 subdomains, which contain the invariant residues predicted to be essential for catalysis and kinase function. Despite the homology of this region among kinases, highly selective compounds targeting specific disease-relevant kinases have recently progressed to marketed drugs.

To facilitate kinase drug discovery efforts, we have developed a premier kinase biology platform to address target access, assay development and high-throughput screening (HTS), lead profiling, cell-based validation, and more. Get unmatched power with our comprehensive portfolio of products and services to investigate the human kinome. You can count on us for proteins, antibodies, biochemical and cellular assays, and everything in between. With four fluorescent biochemical assay formats, over 300 human kinases, and more than 50 cell lines covering 19 pathways, we can help you find the right solution. If that's not enough, our profiling and custom services are always available to help you break through bottlenecks.

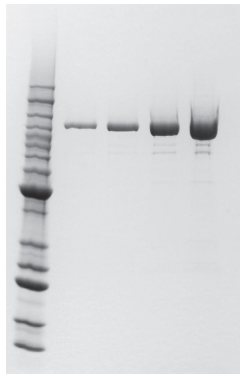
Access our market-leading portfolio of ready-to-use human kinases

Quality and validation are the primary characteristics of our purified recombinant human kinases, ensuring reliability for your disease research and drug discovery. We have established a strictly controlled validation process to maintain quality, consistency, and reproducibility (Figure 1). Each enzyme is:

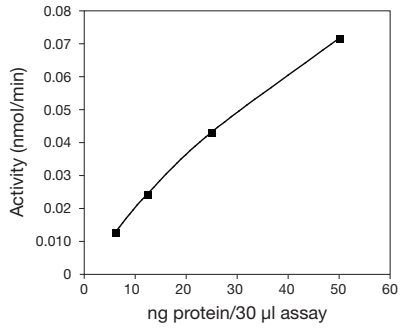
- → Sequence validated prior to expression
- → Expressed according to strictly controlled processes
- → Evaluated by SDS-PAGE for purity
- → Identity confirmed by mass spectrometry
- → Assessed for activity in a radiometric phosphorylation assay

Our kinase target portfolio is designed to meet your specific needs. We provide pre-packed sample vials for small-scale studies or assay development, and larger quantities to support lead discovery programs. If you require specific mutations or orthologs not available in our catalog, we can generate kinases to fit your needs through our Custom Protein Production Services.

A



B



C

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  1  MAPILGYWKI  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  IVGN IKBKE
  1  ----------  ----------  ----------  ----------  ----------  ----------  ----------  ----------  ----------  ----------  NP_054721.1

101  DIRYGVSRIA  YSKDFETLKV  DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK  KRIEAIPQID  KYLKSSKYIA
101  DIRYGVSRIA  YSKDFETLKV  DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK  KRIEAIPQID  KYLKSSKYIA
  1  ----------  ----------  ----------  ----------  ----------  ----------  ----------  ----------  ----------  ----------

201  WPLQGWQATF  GGGDHPPKSD  LVPR
201  WPLQGWQATF  GGGDHPPKSD  LVPRHNQTSL  YKKAGTMQST  ANYLWHTDDL  LGQGATASVY  KARNKKSGEL  VAVKVFNTTS  YLRPREVQVR  EFEVLRKLNH
  1  ----------  ----------  ----------  ------MQST  ANYLWHTDDL  LGQGATASVY  KARNKKSGEL  VAVKVFNTTS  YLRPREVQVR  EFEVLRKLNH

224
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365  VSAQHIAHTT  ASSPLTLFST  AIPKGLAFRD  PALDVPKFVP  KVDLQADYNT  AKGVLGAGYQ  ALRLARALLD  GQELMFRGLH  WVMEVLQATC  RRTLEVARTS

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465  LLYLSSSLGT  ERFSSVAGTP  EIQELKAAAE  LRSRLRTLAE  VLSRCSQNIT  ETQESLSSLN  RELVKSRDQV  HEDRSIQQIQ  CCLDKMNFIY  KQFKKSRMRP

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565  GLGYNEEQIH  KLDKVNFSHL  AKRLLQVFQE  ECVQKYQASL  VTHGKRMRVV  HETRNHLRLV  GCSVAACNTE  AQGVQESLSK  LLEELSHQLL  QDRAKGAQAS

224
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665  PPPIAPYPSP  TRKDLLLHMQ  ELCEGMKLLA  SDLLDNNRII  ERLNRVPAPP  DV
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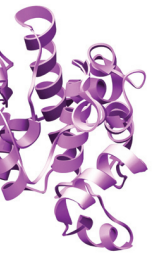
Figure 1—Sample validation data for IKBKE (IKKe). Invitrogen kinases have the Human Genome Nomenclature Committee (HGNC) name in the first position, and the "common" name in parentheses where appropriate. **A.** Analysis of protein preparation by SDS-PAGE. Left to right lanes: BenchMark™ Protein Ladder, 0.5 µg, 1 µg, 2.5 µg, and 5 µg IKBKE (IKKe). **B.** Activity assessment of IKBKE (IKKe) in radioactive assay format. **C.** Sequence alignment of Invitrogen construct with the GenBank® entry. These sequences are 100% identical. For other proteins, differences in the sequences would be indicated by boxes around the differing residues.



Enabling technologies provide robustness and reliability to advance your kinase research

Invitrogen continues to design, develop, and deliver unique and robust assay technologies to meet the demands of the drug discovery community. We have developed a variety of preconfigured fluorescent assay solutions to meet your target validation, assay development, and screening needs.

All Invitrogen kinase assay technologies are nonradioactive, homogeneous, highly miniaturizable, with Z′-factors >0.5, and are available as kits, custom, or bulk reagents. We offer four unique and robust assay technologies optimized for high-throughput screening, including:

- → LanthaScreen™ TR-FRET Assay Development Toolbox
- → Adapta™ Universal Kinase Assay
- → Z′-LYTE® FRET Assays
- → Omnia® Real-Time Kinetic Assays

Use the kinase assay comparison guide to help you select the right Invitrogen technology for your research goals (Table 1).

Table 1— Kinase assay comparison guide.

Application	Primary screening
	LanthaScreen™ assay
	Ideal primary screening technology due to lack of compound interference; universal tyrosine kinase assay; tested with over 200 kinases
Workflow application	High-throughput screening/profiling
Total assay volume (kinase and detection)	20 µl
Ratiometric readout	Yes
Detection technology	TR-FRET
Increase-in-signal assay	Yes
Kinetic vs. endpoint readout	Endpoint
Antibody-based assay	Yes
Detection step required	Yes (antibody)
Reaction time	30–60 min
Assay stability/ read window	Stable for up to 18 hr
Kinases tested	See www.invitrogen.com/lanthascreen
Overcoming interference	Time-resolved readout allows read after interfering signals have decayed
ATP concentration flexibility	Complete flexibility; tested up to 1 mM
Substrate concentration flexibility	100 nM–1 µM
Compatibility with protein substrates	Yes
Compatibility with cell lysates	Yes
Instruments validated by Invitrogen	Tecan GENios™ pro, Tecan Ultra Evolution™, BMG Labtech PHERAstar, Molecular Devices Analyst® HT, PerkinElmer EnVision™, Tecan Safire^{2}™, Molecular Devices SpectraMax® M5

Primary or secondary screening of difficult targets	Secondary screening	Mechanistic studies
Adapta™ assay	**Z´-LYTE® assay**	**Omnia® assay**
Ideal for screening when the target of interest does not phosphorylate traditional peptide substrates; validated specifically for lipid kinase projects	Ideal for secondary screening, hit validation, and profiling because this technology has been validated with >265 kinases; primary technology platform for SelectScreen™ Kinase Profiling Service	Ideal for mechanistic studies and measuring kinase activity in cell lysates; simple mix-and-read fluorescence-based assay allows measurements in real time (kinetics)
High- to medium-throughput screening projects/ secondary screening/profiling	Medium-throughput screening/secondary screening/assay development/profiling	Downstream of high-throughput screening
15 µl	20 µl	20–100 µl
Yes	Yes	No
TR-FRET	FRET	Fluorescence intensity
No	No	Yes
Endpoint	Endpoint	Kinetic
Yes	No	No
Yes (antibody)	Yes (protease)	No
30–60 min	2 hr	Seconds to 120 min
Stable for up to 18 hr	4–18 hr (kit-dependent)	Up to 2 hr (real-time measurement)
See www.invitrogen.com/adapta	See www.invitrogen.com/zlyte	See www.invitrogen.com/omnia
Time-resolved readout allows read after interfering signals have decayed, red Alexa Fluor® dye as acceptor further enhances ability to overcome interference	Preread at 445/520 nm	Kinetic, real-time measurement
Tested from 1 to 500 µM	Complete flexibility; tested up to 1 mM	Complete flexibility; tested up to 1 mM
Complete flexibility, must be greater than ATP concentration	2 µM only	Complete flexibility
Yes	No	No
No	No	Yes
Tecan GENios™ pro, Tecan Ultra Evolution™, BMG Labtech PHERAstar, Molecular Devices Analyst® HT, PerkinElmer EnVision™, Tecan Safire²™, Molecular Devices SpectraMax® M5	Tecan Infinite F-500, Tecan Safire2, BMG Labtech Pherastar	Molecular Devices SpectraMax® M5, Molecular Devices Gemini™ EM, PerkinElmer VICTOR™ Light, PerkinElmer EnVision™, PerkinElmer HTS 7000, BMG Labtech FLUOstar OPTIMA, Tecan Safire

LanthaScreen™ technology—a powerful toolbox for kinase assay development

Every assay application has its own specific requirements. To get the assay components you need without the ones you don't, our LanthaScreen™ reagents are available as stand-alone products. The LanthaScreen™ toolbox for kinase assays includes:

- → Terbium-labeled phosphospecific antibodies
- → Fluorescein-labeled peptide substrates
- → GFP- or fluorescein-labeled protein substrates
- → Terbium-labeled anti-epitope and secondary antibodies

LanthaScreen™ TR-FRET (time-resolved fluorescence resonance energy transfer) detection uses a lanthanide chelate rather than a more traditional organic fluorophore to measure interactions between various binding partners. Terbium (Tb) chelates are readily excited using a 340/35 nm bandpass filter in a standard microtiter plate reader. The emission spectrum of Tb displays four sharp, distinct peaks centered at 490, 546, 585, and 620 nm, with clear baseline separation and "silent" regions between the peaks. Energy transfer to a suitable acceptor, such as fluorescein, can be readily detected in these otherwise silent regions by monitoring an increase in acceptor fluorescence intensity (Figure 2).

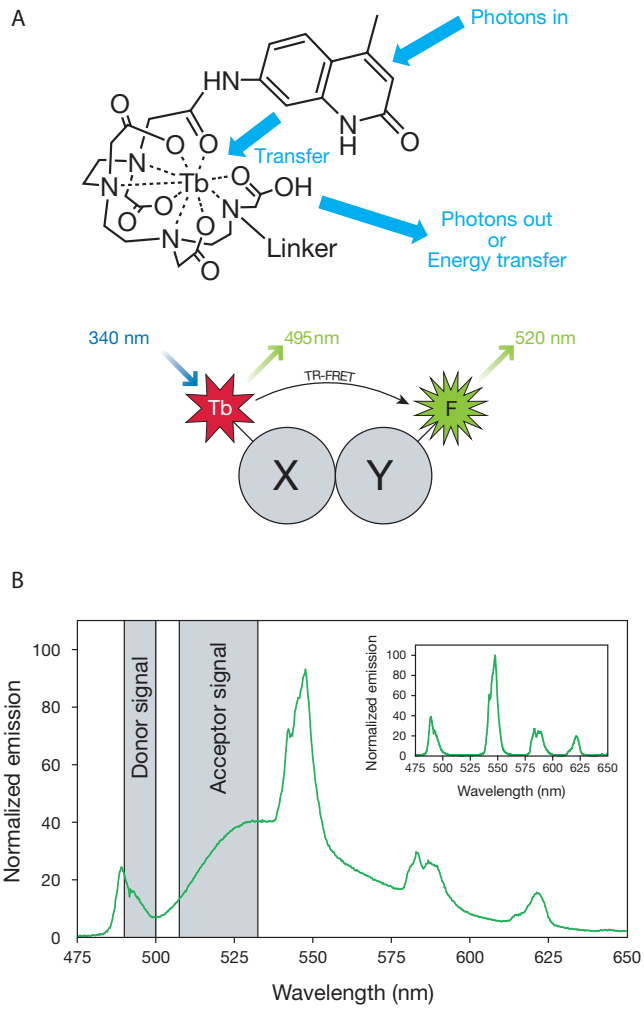


Figure 2—The LanthaScreen™ technology mechanism. **A.** The LanthaScreen™ format is based on the use of a long-lifetime Tb chelate as the donor species and fluorescein as the acceptor species. When Tb- and fluorescein-labeled molecules are brought into proximity, energy transfer takes place, causing an increase in acceptor fluorescence and a decrease in donor fluorescence. These fluorescent signals can be read in a time-resolved manner to reduce assay interference and increase data quality. **B.** The time-resolved spectrum illustrates energy transfer occurring when Tb and fluorescein are brought into proximity via biomolecular interactions. The TR-FRET value is determined as a ratio of the FRET-specific signal measured with a 520 nm filter to the terbium-specific signal measured with a 495 nm filter. The inset shows the time-resolved spectrum in the absence of energy transfer.

LanthaScreen™ TR-FRET assays offer distinct advantages over other fluorescent assay formats, particularly when compound autofluorescence or precipitation are issues. Because the donor species used in our TR-FRET assays has a fluorescent lifetime many orders of magnitude longer than background fluorescence of scattered light or autofluorescent compounds (Figure 3), energy transfer can be measured after the interfering signal has completely decayed (Figure 4).

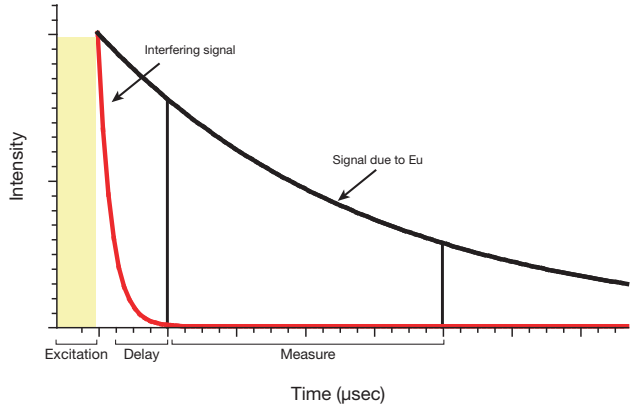


Figure 4—Schematic of measurement setup for detection of TR-FRET in HTS assays. The LanthaScreen™ format is based on the use of a long-lifetime Tb chelate as the donor species and fluorescein as the acceptor species. When Tb- and fluorescein-labeled molecules are brought into proximity energy transfer takes place, causing an increase in acceptor fluorescence and a decrease in donor fluorescence. These fluorescent signals can be read in a time-resolved manner to reduce assay interference and increase data quality.

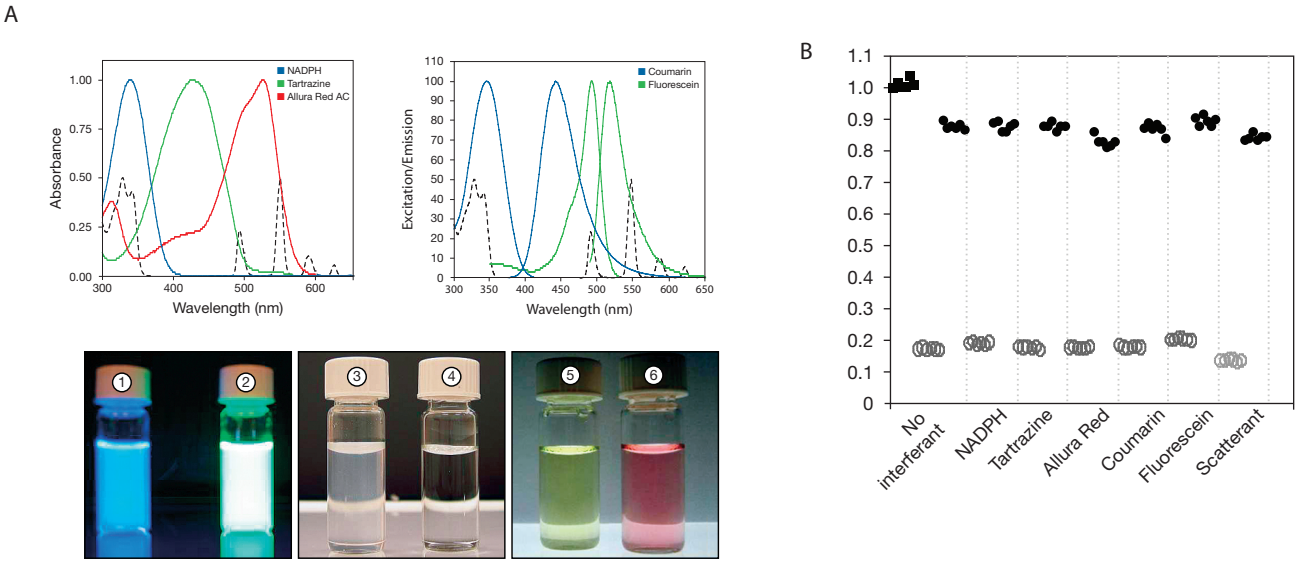


Figure 4—Resistance to interference from color quenchers, fluorescent compounds, and scattered light. A. To demonstrate the resistance of the LanthaScreen™ format to interference from color quenchers, fluorescent compounds, or light scatterants, kinase assays were read in the presence of such compounds. The color quenchers and fluorescent compounds were chosen to overlap with the excitation and emission spectra of the Tb chelates, in order to provide a worst-case scenario for interference. The absorbance or fluorescent spectra of these compounds are shown, along with photos demonstrating the appearance of the solutions in the assay wells. 1: 100 nM coumarin; 2: 100 nM fluorescein; 3: 500 µg/ml nondairy creamer; 4: buffer blank; 5: 10 µM tartrazine; 6: 10 µM Allura Red AC. **B.** Src kinase was assayed in the absence and presence of interfering compounds. Any interference seen in the raw data was compensated for by the ratiometric nature of the readout.

In a LanthaScreen™ kinase assay, a Tb-labeled phosphospecific antibody binds the phosphorylated fluorescein-labeled substrate, resulting in an increased TR-FRET value (Figure 5). The amount of antibody that binds to the substrate is proportional to the amount of phosphorylated substrate present. In this manner, kinase activity can be detected and measured by an increase in the 520 nm/495 nm emission ratio.

As an extension to our LanthaScreen™ peptide-based substrates, we offer physiological protein substrates that are directly labeled with either GFP or fluorescein (Figure 6). These assays function using the same mechanism as the peptide-based substrate assay, but allow LanthaScreen™ assays to

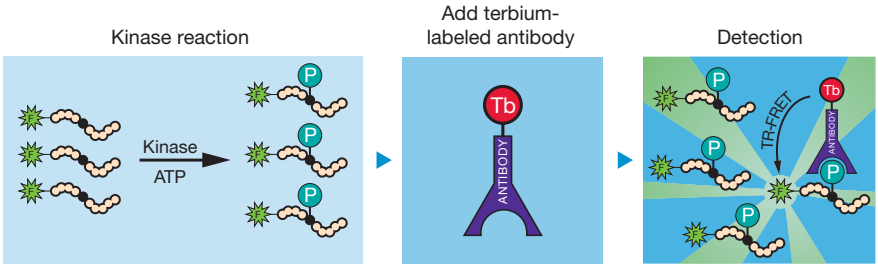


Figure 5—LanthaScreen™ kinase assay using traditional peptide substrates. A fluorescein-labeled kinase substrate peptide is incubated with kinase and ATP. Tb-labeled antibody is then added and phosphorylation is detected by an increase in the TR-FRET ratio. Because the substrate is directly labeled, there is no need to add streptavidin-APC. Additionally, unlike some europium-based systems, there is no requirement to add high concentrations of potassium fluoride, which can potentially disrupt antibody–product interactions.

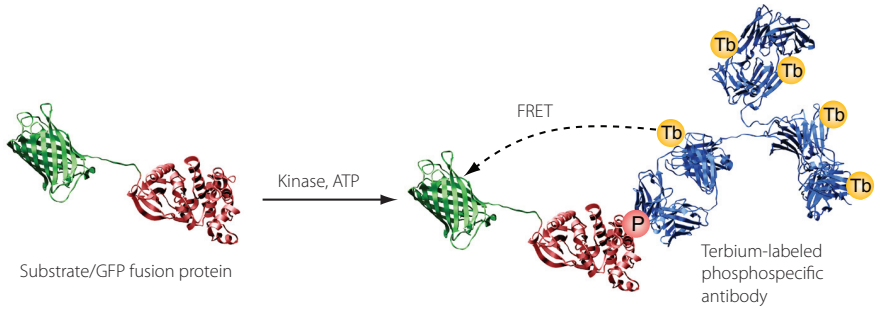


Figure 6—LanthaScreen™ kinase assay using physiological protein substrates. In this example, a GFP-tagged protein substrate is incubated with the test kinase and ATP. A Tb-labeled phosphospecific antibody is then added to detect the phosphorylated product.

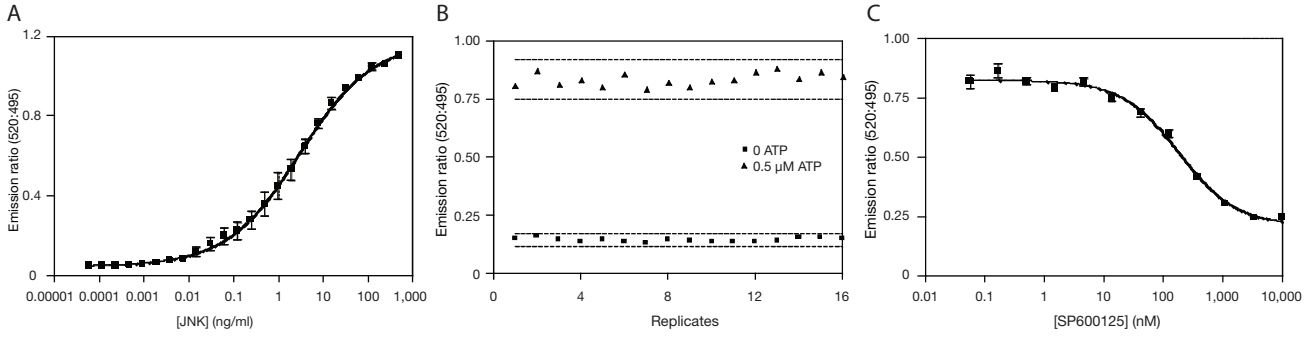


Figure 7—Sample validation data for a LanthaScreen™ kinase assay using a physiological protein substrate. Representative data from the Tb–anti-pATF2 [pThr71] antibody and GFP-ATF2 (19–96) using JNK1 (Cat. no. PV3319). **A.** A two-fold serial dilution of JNK1 kinase with an EC_{80} value of 40 ng/ml. **B.** Data from 16 positive and 16 negative wells give a Z´-factor of 0.84. The dashed lines represent ±3 standard deviations. **C.** Three-fold serial dilution of the JNK1 inhibitor SP600125 assayed against 40 ng/ml JNK1 in the presence of 0.5 µM ATP.

be developed for kinases that preferentially or exclusively act on the physiological protein rather than peptide substrates (Figure 7). See the LanthaScreen™ assay conditions for your kinase at www.invitrogen.com/lanthascreenkinase.

LanthaScreen™ technology has been validated on multiple instrumentation platforms, as described in Tables 1 and 2. Filter sets for filter-based instruments are available from Invitrogen using the part numbers indicated in Table 1. As they become available for each instrument, setup instructions can be found at www.invitrogen.com/lanthascreen.

Excitation filters

We recommend using a 340 nm excitation filter with a 30 nm bandwidth. Other filters with similar specifications may also perform well.

Emission filters

We recommend using a 495 nm emission filter with a 10 nm bandwidth to detect the terbium (donor) emission, and a 520 nm emission filter with a 25 nm bandwidth to detect the fluorescein (acceptor) emission.

The specifications of the 520 nm filter are more critical than those of the excitation filter. In general, standard "fluorescein" filters may not be used, because such filters also pass light associated with the terbium spectra as well.

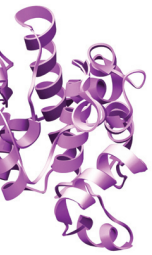
Table 1—Filter- and CCD-based instruments compatible with LanthaScreen™ technology.

Manufacturer	Instrument	Excitation filter part number	Emission filter set part number	Recommended dichroic mirror
PerkinElmer	EnVision™	PV00215	PV00315	LANCE™ dual
PerkinElmer	VICTOR™	Preinstalled	PV00325	Not applicable
Molecular Devices	Analyst®	PV00225	PV00325	380 or 400
Tecan	Infinite™ F500	PV002125	PV003125	505 (fluorescein)
Tecan	Ultra™	PV002125	PV003125	505 (fluorescein)
Tecan	GENios Pro™	PV002125	PV003125	505 (fluorescein)
BMG Labtech	PHERAStar™	(contact BMG Labtech)		
Perkin Elmer	ViewLux	(contact PerkinElmer)		

Table 2—Monochrometer-based instruments compatible with LanthaScreen™ technology.*

	Tecan Safire2™	Molecular Devices M5
Excitation wavelength	332/20 nm	332/15 nm
Emission wavelength, donor	485/20 nm	488/15 nm
Emission wavelength, acceptor	515/20 nm	518/15 nm
Cutoff	NA	420 nm

* Please note that when using a monochrometer-based instrument, white assay plates often show better performance.



LanthaScreen™ kinase assay FAQs

Which LanthaScreen™ antibody should I use in my kinase assay?

For tyrosine kinase assays, a Tb-PY20 antibody is available. Performance of this antibody is excellent when using fluorescein-labeled Poly-GT or Poly-GAT as the substrate. For Ser/Thr kinases, the performance of various kinases using a suitable antibody is shown in a reactivity chart at www.invitrogen.com/lanthascreenkinase.

How much antibody should I use for my kinase assays?

In general, we recommend 2 nM as the final concentration of antibody in the detection reaction, although less may be used. The molecular weight of a Tb-labeled antibody is 150,000, so a concentration of 2 nM corresponds to 0.3 µg/ml.

How many assays can I run with a given amount of antibody?

This depends on the volume of the assay and the concentration of antibody used. Using 2 nM antibody in a final volume of 20 µl, 25 µg of antibody will run >4,000 assays and 1 mg of antibody will run >165,000 assays.

How much peptide substrate should I use in a LanthaScreen™ assay?

In general, 200 nM to 1 µM peptide substrate is used in a LanthaScreen™ kinase assay. We recommend starting with 200–400 nM in the assay reaction. The micromolar concentration of the substrate can be calculated from the molecular weight. Divide the substrate concentration (in mg/ml) by the molecular weight, then multiply by 10^6 to obtain the concentration in µM.

Which substrate should I use to assay my tyrosine kinase?

The choice between Poly-GT or Poly-GAT depends upon your kinase. Two synthetic peptides determined to be effective substrates for src family kinases, peptide 1 and peptide 2, are currently available. Peptide 1 is a random peptide identified to be a pp60c-Src substrate, and peptide 2 is a sequence from p34cdc2. Alternatively, fluorescein-labeled peptide substrates can be readily synthesized by Invitrogen to suit your particular needs. In addition to peptide substrates, we offer fluorescein-labeled Poly-GT and Poly-GAT. In general, either substrate will work for any given tyrosine kinase. However, one of the substrates will probably show a lower EC_{50} with the kinase, allowing less kinase to be used in the assay. Additionally, one substrate may show a larger assay window than the other, but either substrate will give an assay with an excellent Z′-factor. To date, these substrates have a 100% success rate for assaying tyrosine kinases.

I already have a biotinylated substrate I know works with my kinase. Can I use this in a LanthaScreen™ assay?

Yes, a biotinylated substrate can be used in a LanthaScreen™ assay, provided you have fluorescein-labeled streptavidin (Cat. no. SA10002). A typical assay should contain 200–400 nM biotinylated substrate in the kinase reaction, 1–2 nM Tb-labeled antibody, and 50 nM fluorescein-labeled streptavidin to detect the product. The conditions should be optimized for your particular system, but these recommended concentrations are a good starting place. This format does, however, increase the cost associated with screening, due to the need to purchase an additional component. Also, it adds another layer of complexity with the optimization required for the additional component.

How many assays can I run with a given amount of peptide substrate?

The number of assays you can run depends on the concentration of peptide substrate used in the assay. For most peptide substrates, 1 mg of peptide will run approximately 250,000 wells (10 µl reaction, 200 nM peptide). For Poly-GT or Poly-GAT, the 1 ml, 30 µM size will run approximately 16,700 wells (10 µl reaction, 200 nM substrate).

I already have an antibody that I would like to use in a LanthaScreen™ kinase assay. How can I determine if it will work without directly labeling it?

You may "indirectly" label your primary antibody using a Tb-labeled, species-specific anti-IgG antibody. As a starting point, we recommend a final concentration of 2 nM primary antibody and 5 nM secondary antibody in the assay well. The reaction conditions can be further optimized by increasing or decreasing the amount of Tb-labeled secondary antibody to determine the optimal concentration to use in your particular assay. Check with the supplier of your primary antibody to determine its stock concentration. In the majority of cases, the unlabeled primary and Tb-labeled secondary antibody may be premixed prior to addition to the assay well. In some cases, separate additions are required. In general, assays using directly labeled antibodies come to equilibrium faster or have more stable signals than assays that use indirect labeling techniques. Although the response window (Z'-factor) is typically acceptable when using an indirect labeling strategy, it may be possible to improve the window by using a directly labeled primary antibody.

What filters/dichroic mirrors do I need to read a LanthaScreen™ assay?

We recommend using a 340 nm excitation filter with a 30 nm bandwidth, but an excitation filter with similar specifications will perform well. The exact specifications of the emission filters are more important. In a LanthaScreen™ assay, the donor (Tb) intensity is measured using a 490 or 495 nm filter (10 nm bandwidth), and the fluorescein (acceptor) intensity is measured using a 520 nm filter (25 nm bandwidth). Filters suitable for performing LanthaScreen™ assays can be obtained from Invitrogen or from other vendors. A filter module for the BMG PHERAstar™ can be obtained directly from BMG Labtech.

The dichroic mirror should ideally have a cutoff of less than 490 nm. A dichroic mirror meeting this specification is available from several vendors, including Chroma (www.chroma.com), or from the instrument manufacturer. In some instruments, the fluorescein dichroic performs well but must be manually selected. In other instruments, a 50:50 mirror may be selected but in general will not give as high performance as a dedicated dichroic. The LanthaScreen™ filter module from BMG Labtech contains an optimal dichroic mirror.

Are there specific assay plates I should use with a LanthaScreen™ assay?

The LanthaScreen™ format is suitable for use with nearly any brand or volume of assay plate. Invitrogen has developed many assays using Corning® low-volume black polystyrene 384-well plates (Corning Life Sciences, Cat. no. 3676). We have also successfully used Corning® low-volume white polystyrene plates (Corning Life Sciences, Cat. no. 3673), Thermo LabSystems Microfluor 1 Black 96-well plates (Thermo Scientific, Cat. no. 7005), and Thermo Electron Cliniplate black polypropylene 384-well plates (Thermo Scientific, Cat. no. 95040020). When performing the assay on monochromator-based readers, assay performance may be increased by using white plates. With filter-based instruments, differences between black and white plates are often negligible.

For more information on our LanthaScreen™ assay technology, or to find the assay conditions for your kinase of interest, visit www.invitrogen.com/lanthascreen.

Adapta™ Universal Kinase Assay and Substrates

- → High Z´-factors at low percent conversion of ATP to ADP using less kinase than ATP depletion assays
- → One assay suitable for all kinases, including difficult-to-assay targets such as lipid kinases
- → Best-in-class Alexa Fluor® dyes enable a superior red-shifted TR-FRET assay
- → Performance validation using our portfolio of specialized substrates and optimized assay buffers

The Adapta™ Universal Kinase Assay uses time-resolved fluorescence resonance energy transfer (TR-FRET) to monitor ADP formation as the kinase assay progresses. TR-FRET detection technology utilizes a lanthanide chelate rather than a more traditional organic fluorophore to measure interactions between binding partners. As with other TR-FRET systems, the Eu (europium) donor is excited using a 340 nm excitation filter with a 30 nm bandpass. Eu chelates can effectively conduct energy transfer to a range of suitable far-red acceptors, such as Alexa Fluor® 647, that are readily detected in the silent regions between the Eu emission peaks. Following energy transfer, the emission from the Alexa Fluor® 647 can be measured using a filter centered at 665 nm with a 10 nm bandpass. This signal is then referenced (or "ratioed") to the emission from the Eu, which is detected using a 615 nm filter with a 10 nm bandpass. The "emission ratio" is calculated as the 665 nm signal divided by the 615 nm signal.

The Adapta™ assay can be divided into two phases: a kinase reaction phase and an ADP detection phase (Figure 1). In the kinase reaction phase, all kinase reaction components are added to the well and the reaction is allowed to incubate for a set period of time, typically 60 minutes. After the reaction, a detection solution of Eu-labeled anti-ADP antibody, Alexa Fluor® 647–labeled ADP tracer, and EDTA (to stop the kinase reaction) are added to the assay well. ADP formed by the kinase reaction will displace the Alexa Fluor® 647–labeled ADP tracer from the antibody, resulting in a decrease in TR-FRET signal. In the presence of an inhibitor, the amount of ADP formed by the kinase reaction is reduced, and the resulting intact antibody–tracer interaction maintains a high TR-FRET signal.

The Adapta™ Universal Kinase Assay measures kinase activity by detecting ADP formation. Most of the signal change in the

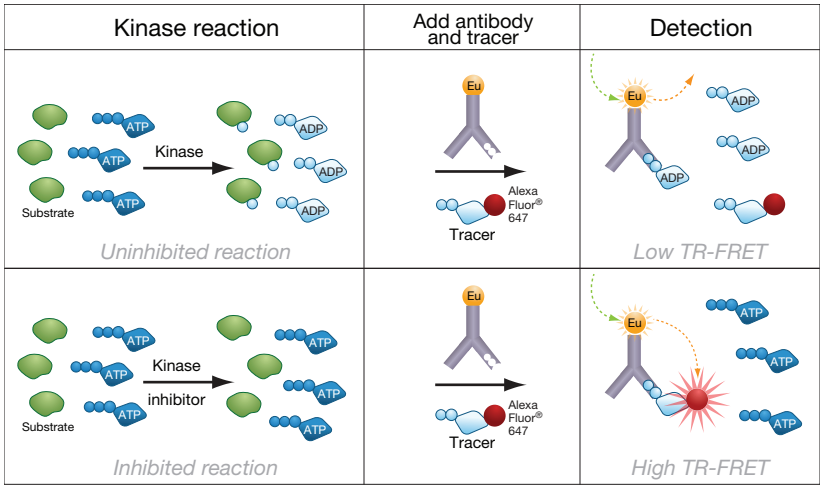


Figure 3—Schematic of the Adapta™ Universal Kinase Assay.

assay occurs in the first 10–20% of conversion of ATP to ADP (Figure 2). This is in sharp contrast to kinase assays that measure ATP depletion, in which 20% conversion of ATP to ADP results in only a 20% signal change. As a result, the Adapta™ Universal Kinase Assay produces high Z´-factors at low percent ATP conversion (Figure 3). Because reduced amounts of kinase are required to achieve an optimal assay window, the assay is ideal for kinases with lower activity.

The Adapta™ Universal Kinase Assay has been successfully tested with both lipid kinases (Figure 4) and protein kinases (Figure 5). The experimental results obtained demonstrate a clear correlation with the established pharmacology of the kinases and inhibitors tested.

In addition to the Adapta™ Universal Kinase Assay Kit, we offer a selection of lipid- and peptide-based substrates for use with the Adapta™ assay. Both the lipid and peptide substrates were designed and optmized with assay performance in mind. Our lipid substrates were designed for optimal performance in the Adapta™ Universal Kinase Assay with our PI3 kinases. No rehydration, sonication, or extrusion needed—the lipid substrates are ready for imme-

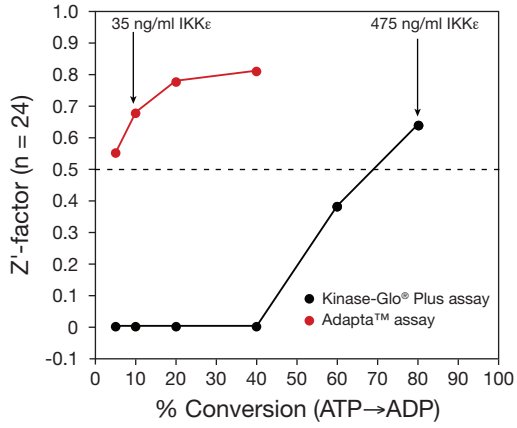


Figure 5—Comparison of Z´-factors for the Adapta™ Universal Kinase Assay and Kinase-Glo® Plus Luminescent Kinase Assay. Due to the sensitivity of the Adapta™ Universal Kinase Assay to small changes in ADP formation, Z´-factors of ≥0.5 are achieved with as little as 5% conversion of ATP to ADP. For the Kinase-Glo® Plus Luminescent Kinase Assay (Promega Corp.), a much higher conversion (~80%) is necessary to achieve similar Z´-factors. This makes the Adapta™ assay ideally suited for use with kinases with lower activity, since less kinase has to be used to achieve an optimal assay window.

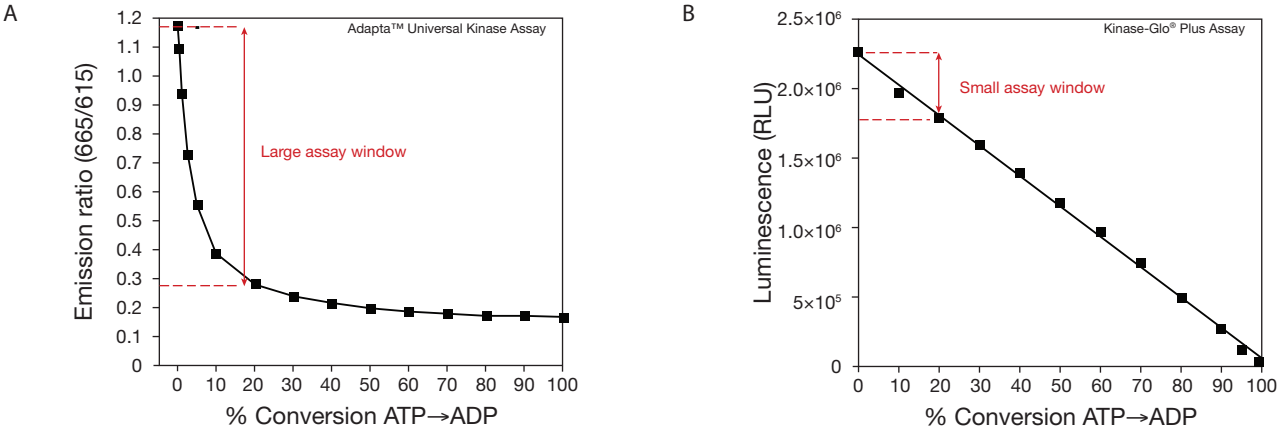


Figure 4—Assay signal as a function of percent conversion of ATP to ADP. A. A representative ATP→ADP titration curve highlights the large change in assay signal in the Adapta™ assay at only 20% conversion of ATP to ADP. **B.** In a typical ATP depletion assay (Kinase-Glo® Plus Luminescent Kinase Assay, Promega Corp.), the relative change in the assay window at 20% conversion is considerably smaller.

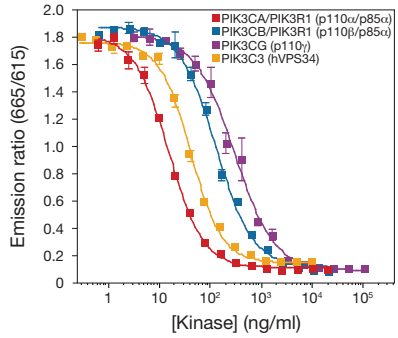
diate use in your kinase assay. The substrates are formulated for optimal performance with PI3 kinases, and allow you to get reliable, reproducible performance each and every time, even after multiple freeze-thaw cycles.

Since lipid kinases are often highly sensitive to reaction conditions, such as the type and concentration of detergents and salts, we have optimized buffers for each PI3 kinase in our collection.

A growing collection of Invitrogen kinases have been validated for use with the Adapta™ Universal Kinase Assay Kit and corresponding substrates. To see if your kinase has been validated with the Adapta™ assay, visit www.invitrogen.com/adapta.

Most instruments, settings, and filters that work with other Eu-based TR-FRET assay systems can be used with the Adapta™ Universal Kinase Assay. For assistance in determining if your instrument is suitable to read this assay, or for assistance with instrument setup, please call us in North America at +1 760 603 7200 (select option 3, then enter ext. 40266).

A



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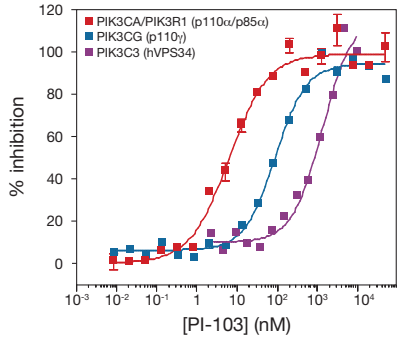


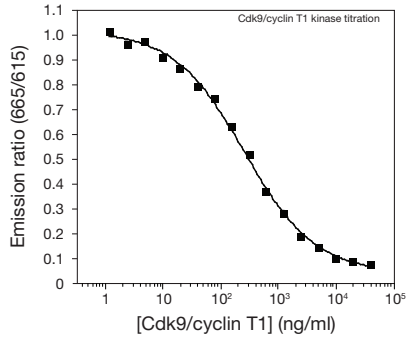
C

Kinase	Inhibitor	IC_{50} (nM) Literature*	Observed
PIK3CA/ PIK3R1 (p110α/ p85α)	PI-103	8	6.3
PIK3CG (p110γ)	PI-103	150	89.2
PIK3C3 (hVPS34)	PI-103	2,300	1,324

* Knight, Z.A. et al. (2006) *Cell* 125 (4):733-747.

Figure 6—Assaying class I and class III lipid kinases. The Adapta™ Universal Kinase Assay can be used to assay both class I and class III PI3 kinases. The performance of the Adapta™ assay with some common PI3K lipid kinases **(A)** was examined. Inhibitor titrations performed with PI-103 **(B)** display the anticipated pharmacology **(C)**.

A



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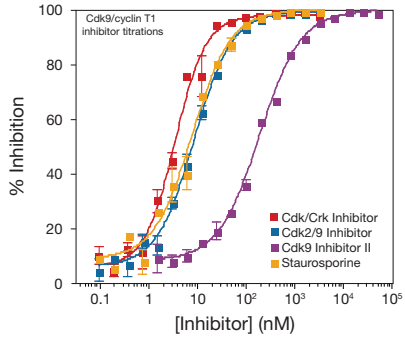


C

Kinase	Inhibitor	IC_{50} (nM) Literature*	Observed
Cdk9/cyclin T1	Cdk/Crk Inhibitor	6–10	3.1
	Cdk2/9 Inhibitor	4	7.7
	Cdk9 Inhibitor II	350	182.1
	Stauro-sporine	NR	5.7

NR = No value reported. * Caligiuri, M. et al. (2005) *Chem Biol* 12:1103-1115; Chao, S. et al. (2000) *J Biol Chem* 275:28345-28348; Wang, S. et al. (2004) *J Med Chem* 47:1662-1675; Krystof, V. et al. (2006) *J Med Chem* 49:6500-6509.

Figure 7—Assaying protein kinases. In addition to lipid kinases, the Adapta™ Universal Kinase Assay can be used to identify inhibitors for difficult or low-activity protein kinases. For example, following a titration of Cdk9 with the Adapta™ assay **(A),** known Cdk inhibitors were evaluted **(B).** The resulting IC_{50} values have a strong correlation to literature values **(C)**.

Simple ratiometric inhibitor screening with the Z′-LYTE® FRET-based kinase assay platform

- → Nonradioactive, antibody-independent format saves time and money while providing a safer assay
- → Flexible design allows you to vary ATP levels to detect your compound's mechanism of action
- → Ratiometric data readout, excellent Z′-factors (>0.5), and performance-validated reagents lead to accurate results

Fluorescence resonance energy transfer (FRET)-based Z′-LYTE® assays do not require expensive radioactive substrates or antibodies. They are compatible with automated high-throughput screening (HTS) systems and can be completed in less than three hours, providing you with quick selectivity profiling results. Normalized results and high Z′-factors supply the accuracy and consistency you need to identify hits.

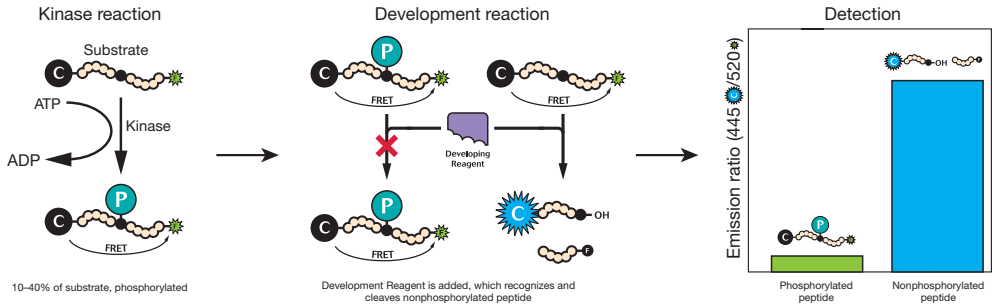


Figure 12—Schematic of the Z′-LYTE® assay. The kinase transfers the γ-phosphate of ATP to a single Ser, Thr, or Tyr residue in the synthetic peptide substrate (2 µM). The peptide is labeled with two fluorophores (coumarin and fluorescein)—one at each end, to make up a FRET pair. In the development reaction, a site-specific protease recognizes and cleaves nonphosphorylated peptides. Phosphorylation of peptides suppresses cleavage by the protease. Cleavage disrupts FRET between the coumarin and the fluorescein on the peptide, while uncleaved, phosphorylated peptides maintain the FRET signal. The ratiometric readout of the donor emission over the acceptor emission quantitates reaction progress. The ratio is low if the peptide is phosphorylated, and high if the peptide is nonphosphorylated. Compounds that inhibit kinase activity will therefore produce a high ratio, and are easily distinguished from potential protease inhibitors that produce a low ratio.

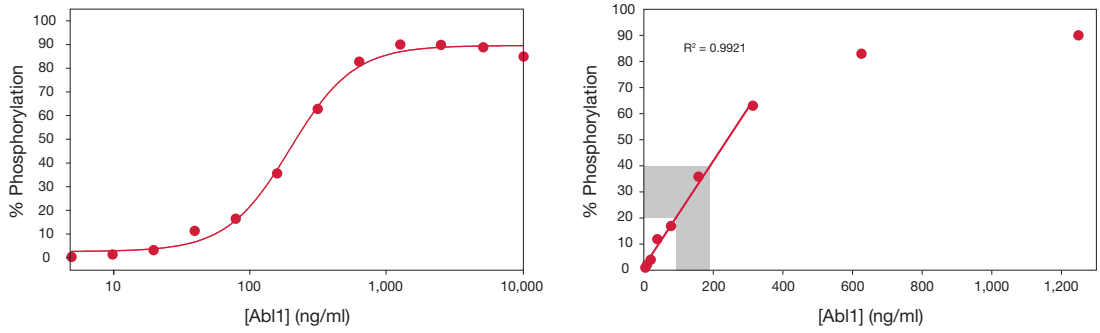


Figure 13—Percent phosphorylation as a function of enzyme concentration. Representative sample data generated for Abl1 for the Z′-LYTE® Kinase Assay Kit - Tyr 2 Peptide. The kinase titration data shown for Abl1 demonstrate that the reaction is linear up to approximately 60% phosphorylation; however, we recommend targeting 20 to 40% phosphorylation for screening, as indicated by the gray shading.

Z′-LYTE® kinase assay kits are suitable for both Ser/Thr and Tyr kinases. Invitrogen has validated over 228 protein kinases using this assay technology, and it is our primary assay platform for our SelectScreen™ biochemical kinase profiling services. This format is based on FRET between coumarin and fluorescein (Figure 12). The ratiometric readout results in a low % CV, allowing for high Z′-factors at low-percent phosphorylation (Figure 13). Complete flexibility in ATP concentrations allows the detection of both ATP-competitive and allosteric inhibitors.

To demonstrate the application of the Z′-LYTE® technology for kinase screening, we screened the LOPAC™ library in a 384-well format for protein kinase A (PKA) and Ser/Thr Peptide 1; inhibitors were flagged as initial hits of >50% inhibition, and follow-up IC_{50} determinations gave appropriate rank order and potency values (Figure 14). Selectivity data obtained using Z′-LYTE® assays show strong correlation with traditional radioactive filter-capture methods (Figure 15), while data are obtained with less expense and effort.

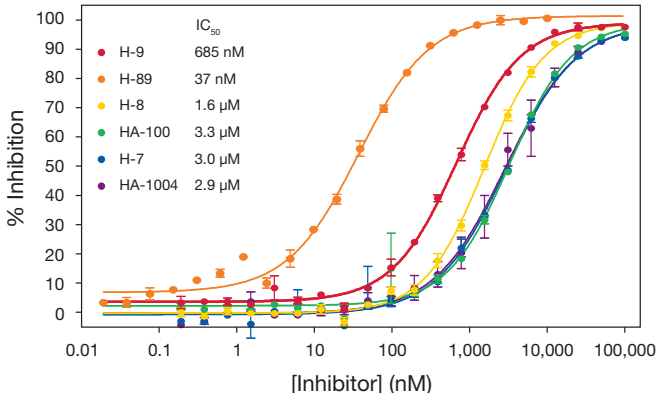


Figure 14—Z′-LYTE® Ser/Thr 1 peptide inhibition assay for PKA. Six inhibitors were assayed against PKA using the Z′-LYTE® Kinase Assay Kit–Ser/Thr 1 Peptide at 10 µM ATP. The inhibitor titration data demonstrate that the assay is able to distinguish very potent inhibitors with IC_{50} values that correlate with published literature values. Z′-factors were calculated based on controls for each assay, and ranged between 0.82 and 0.91.

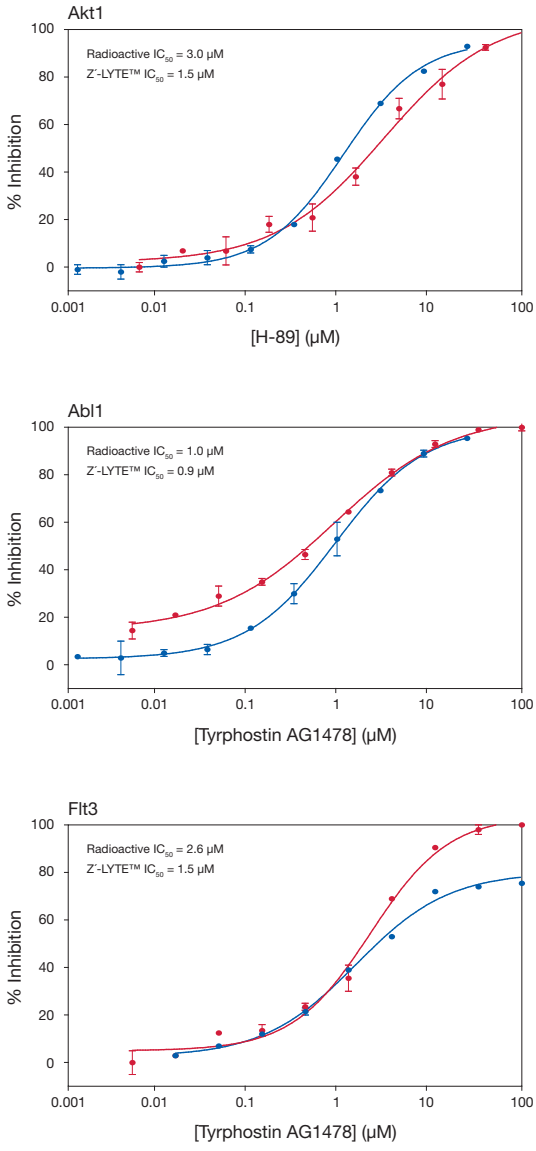


Figure 15—Correlation between Z′-LYTE® assays and radioactive methods. Three kinases were assayed by both the Z′-LYTE® assay and a radioactive filter-capture method in the presence of representative inhibitors. All assays were performed at the $K_{m[app]}$ for ATP as determined for each assay. The Z′-LYTE® assays required less enzyme than the radioactive assays. There is excellent IC_{50} value correlation between the two assay formats.

Table 4—Z′-LYTE®-compatible monochromator-based instruments.

	Tecan Safire and Safire²™
Excitation filter	400/12*
Emission filter, donor	445/12*
Emission filter, acceptor	520/12*

*Wavelength in nm/bandpass

Z´-LYTE® technology can also be used to conduct coupled kinase assays, as an approach for screening inhibitors of upstream kinase targets when peptide substrates are difficult to generate. This allows inhibitors to "see" unactive kinases, which can be beneficial in the identification of type II inhibitors. This is demonstrated in Figure 16, which shows the development and validation of a MAPK14 (p38α)/MAPKAP-K2 pathway assay using the Z´-LYTE® platform. In this assay, active p38α phosphorylates inactive MAPKAP-K2, which in turn phosphorylates the MAPKAP-K2 Z´-LYTE® peptide substrate. Once lead compounds are identified, they are screened against the downstream kinase to ensure that they only inhibit the target kinase.

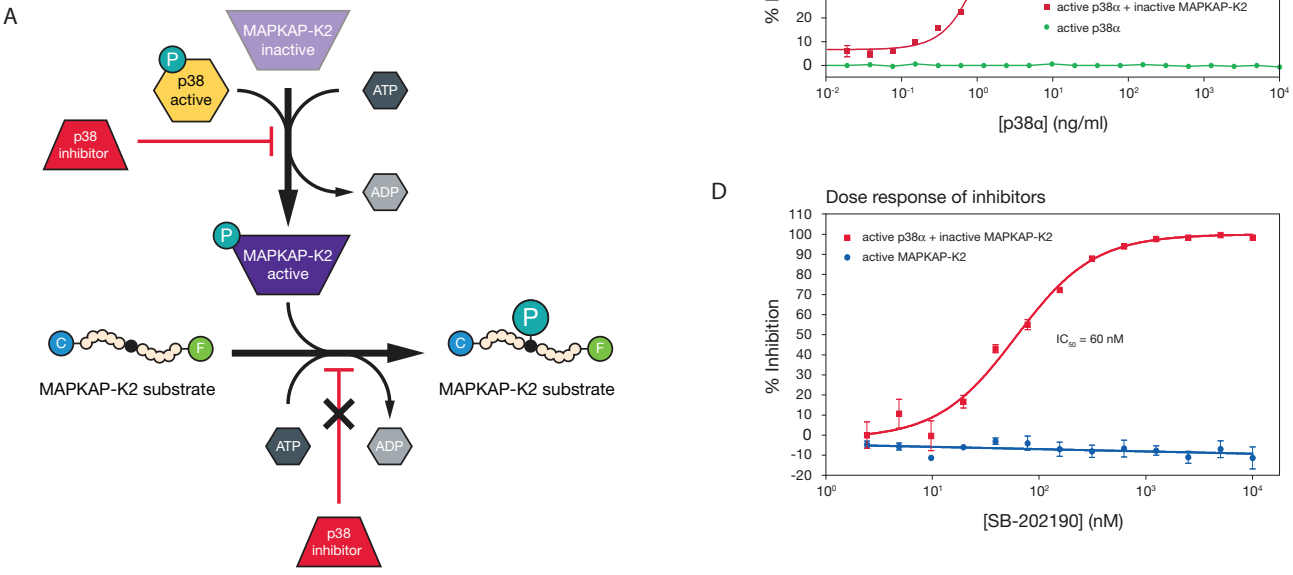


Figure 16—The Z´-LYTE® kinase assay pathway approach. **A.** The Z´-LYTE® p38α pathway assay schematic. **B.** Titration of unactive MAPKAP-K2 in the presence of excess active p38α compared to a titration of active MAPKAP-K2. Overlapping curves indicate integrity of unactive MAPKAP-K2. The concentration of MAPKAP-K2 that resulted in nearly 100% phosphorylation was chosen to be used in the cascade assay. **C.** Active p38α titrated in the absence of unactive MAPKAP-K2. The concentration of active p38α that resulted in approximately 30% phosphorylation was selected to be used in the cascade assay. **D.** A known p38α inhibitor, SB-202190, serially diluted in presence of active p38α and unactive MAPKAP-K2 at the concentrations found in panels B and C. This inhibitor assay yielded the expected value for the IC_{50} of the compound tested, and the inhibitor was not found to inhibit phosphorylation of MAPKAP-K2 substrate by active MAPKAP-K2.

Table 5—Z'-LYTE®-compatible filter-based instruments.

	Tecan GENios Pro™	Tecan Ultra™ series	Molecular Devices Analyst®	PerkinElmer EnVision™
Excitation filter	405/20*	405/20*	405/35*	400/25*
Emission filter, donor	465/35*	465/35*	460/40*	460/25*
Emission filter, acceptor	535/20*	535/20*	530/25*	535/25*
Dichroic for excitation	330/420:440/850	50%	425	General dual
Dichroic for emission	330/420:440/850	320/500:520/800	425	General dual

*Wavelength in nm/bandpass

Measure kinase activity in real time with Omnia® kinase assays

Omnia® kinase assays measure kinase activity in real time, eliminating the need for multiple endpoint assays and saving time, money, and valuable samples. Kinetic data can be collected for specific kinases using recombinant proteins or crude cell lysates. The assays are fast and easy to perform, without radioactivity or specialized equipment. You get the same reliability as from radioactive filter-binding assays, but without the exposure and hassle of hazardous materials. Omnia® kinase assays offer several key advantages:

- → Real-time results—collect kinetic data in one reaction, saving time, money, and sample
- → Flexibility—use recombinant kinases or crude cell lysates
- → Speed—get results in under an hour
- → Minimal interference—overcome false positives or negatives with kinetic format

Omnia® kinase assays utilize a chelation-enhanced fluorophore (CHEF) called Sox, which is incorporated into a kinase-specific peptide substrate to measure phosphorylation (Figure 8). Upon phosphorylation of the peptide by the kinase of interest, there is an increase in fluorescence proportional to the amount of peptide phosphorylation. The substrate-based fluorescence technology allows for rapid, homogeneous, and sensitive detection of enzymatic activity using a simple protocol (Figure 9). Sox technology was developed at MIT and is exclusively licensed to Invitrogen.

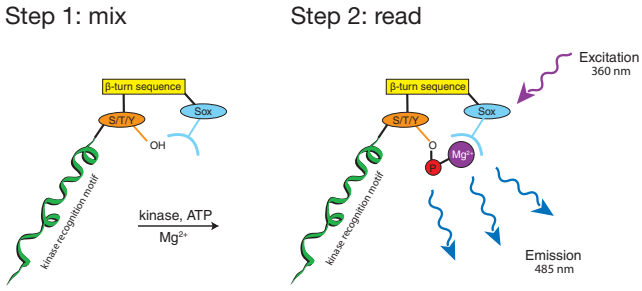


Figure 8—Omnia® kinase assay mix-and-read format. Upon phosphorylation of the substrate, Mg^{2+} is chelated to form a bridge between the Sox moiety and the phosphate group. Excitation at 360 nm results in an increase in fluorescence emission at 485 nm.

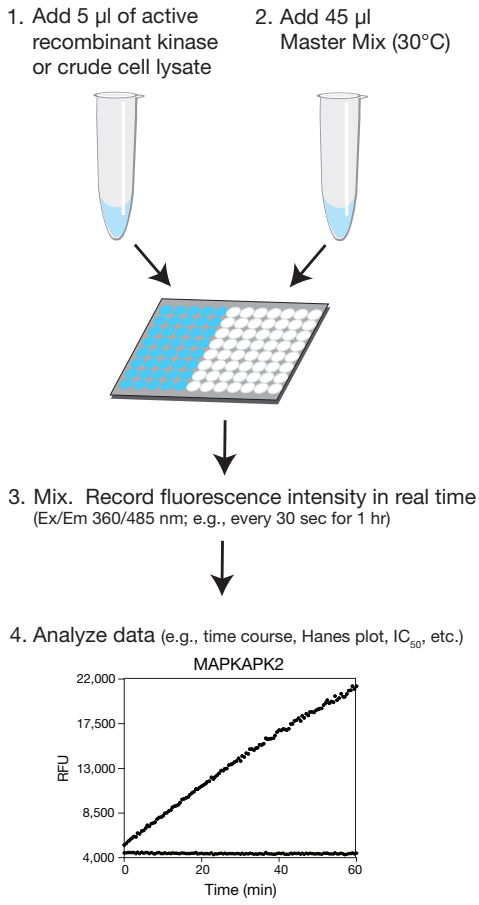


Figure 9—Procedure for Omnia® recombinant and lysate assays. Simply mix the reaction in a 96-well or 384-well plate, and read the fluorescence. Data can be collected every 30 sec for up to 1 hr.

Omnia® kinase assays can be used for a variety of titration studies (ATP, substrate, enzyme, and inhibitor) and for determining K_m, V_{max}, IC_{50}, K_i, K_{on}, and K_{off} values (Figure 10). Due to their compatibility with a wide range of ATP concentrations including physiological ATP (mM), both allosteric and ATP competitive inhibitor studies can be performed. These assays also provide high sensitivity (Figure 11) and precision, which result in excellent Z′-factors (Table 3).

Over 160 Ser/Thr and Tyr kinases have been validated with Omnia® substrates. Several formats are available to meet your needs. Omnia® Recombinant and Lysate assays allow for homogeneous, direct measurements of kinase activity using purified recombinant proteins or crude cell lysates, respectively. Omnia® IP assays, which are available in plate or bead format, utilize an antibody to first capture the kinase of interest from crude cell lysate. Then, the purified kinase is simply mixed with the assay reagents and read in real time. Also available are Omnia® Sampler Plates, which provide a convenient way to identify the best substrate for a particular kinase of interest.

Visit www.invitrogen.com/omnia to learn more about the Omnia® assay format and to find the recommended substrate for your kinase.

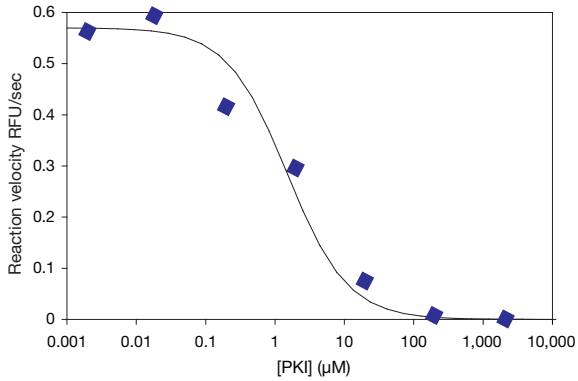


Figure 10—IC_{50} determination in crude cell lysates. Isoproterenol-stimulated LNCaP cell lysates were assayed for PKA activity in the presence of increasing concentrations of PKI-tide. The estimated IC_{50} from this plot is 1 nM.

Table 3—High precision of Omnia® assays. Z′-factors, % CV, and signal-to-background and signal-to-noise ratios for the Omnia® assay for Akt at 10 µM substrate.

% Conversion	Signal-to-background ratio	Signal-to-noise ratio	Z′-factor	% CV
0				4.20
2.5	1.15	2.10	0.67	5.10
5	1.31	4.00	0.77	5.00
10	1.61	6.84	0.82	4.90
15	1.91	7.81	0.78	5.70
20	2.22	12.11	0.88	4.10
50	4.08	18.54	0.88	3.90
100	6.97	18.91	0.86	4.50

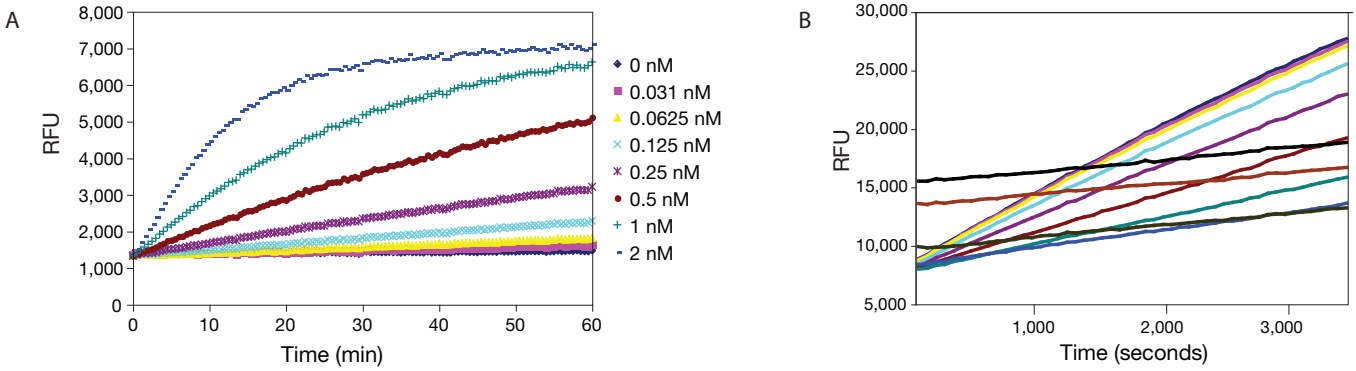
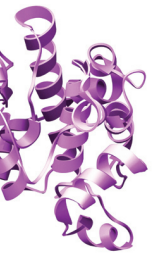


Figure 11—High sensitivity with minimal interference. A. The Omnia® Ser/Thr substrate 8 was used to measure the activity of recombinant PKCα at decreasing protein concentrations. A 20% conversion of substrate to product was achieved at about 200 pM PKCα. **B.** Interference from increasing concentrations of the autofluorescent Akt inhibitor VIII (from 0 to 30,000 µM) is overcome by monitoring the rate of the reaction (change in fluorescent intensity over time) rather than an endpoint fluorescent signal. The IC_{50} value for this inhibitor was determined to be 94 nM.



SelectScreen™ Kinase Profiling Service for library screening or selectivity profiling

Invitrogen's SelectScreen™ Kinase Profiling Service brings together our growing library of purified protein kinase targets and our robust kinase assay technologies to create the premier outsourced service in the market. Obtain rapid profiling of your compounds against a panel of kinases at a single concentration, or choose to have more in-depth IC_{50} determinations performed at variable ATP concentrations. The SelectScreen™ Library Screening Service can rapidly screening a library of compounds including hit confirmation and follow up IC_{50} determinations with any of our validated kinase assays.

The SelectScreen™ profiling services offer you high-quality data, guaranteed two-week turnaround, flexibility in screening parameters, competitive pricing, complete confidentiality, and an open business model with no minimum commitment.

A stringent validation process ensures the highest-quality data possible. Every 384-well plate we screen contains multiple controls that determine whether the plate passes or fails. These include:

- → Duplicate value difference determinations, n = 2
- → Control wells to assess assay interference—protease and fluorescence controls are used to assess assay interference for every compound on every plate
- → Phosphorylation controls
- → Control inhibitor IC_{50} determination for each kinase

Comprehensive titrations are performed for all of our profiling kinases against Invitrogen's panel of substrates. In addition, ATP titrations are performed for each kinase to determine the ATP $K_{m[app]}$ for each target. As a result, you can choose to have your compound run at, above, or below the ATP $K_{m[app]}$ for most kinases in the panel. This flexibility allows you to identify specific types of inhibitors with various mechanisms of action.

We ensure the consistency and reliability of each target. All kinases are of human origin; are quality controlled through mass spectrometry, SDS-PAGE, and radioactivity assays; and are the same proteins sold in the Invitrogen catalog. The kinases on the SelectScreen™ panel have been selected based on therapeutic relevance, pathway biology, and phylogenetic diversity. Visit us at www.invitrogen.com/kinaseprofiling for more information or to request a quotation.

Other kinase biology resources available from Invitrogen

phosphoELISA™ Kits—quantify phosphorylation site–specific proteins

Confirm your western blot data with phosphoELISA™ assays—the easy and unbiased way to identify and quantify phosphorylation site–specific proteins in only 4 hours. Our phosphoELISA™ assays are sandwich ELISAs, utilizing a pan antibody precoated onto a 96-well plate to first capture the total protein, followed by a detector antibody which is phosphosite-specific (i.e., Akt [pS473]). phosphoELISA™ assays measure intracellular proteins accurately with high specificity and sensitivity, allowing for as few as ~4,000 cells/well and/or detection of low-expression proteins.

Invitrogen's phosphoELISA™ Kits contain all the needed reagents: calibrated standard to be used for quantitative results and as positive controls, wash and diluent buffers, antibody-

coated 96-well strip-well plate for flexibility in sample size, detection antibody (phosphospecific or total protein) and HRP-conjugated secondary antibody, stop solution, chromogen, plate covers, and a detailed protocol with kit-specific data.

Please visit www.invitrogen.com/pELISA for current product updates.

CellSensor® Cell Lines—understand entire cell signaling pathways in record time

Our CellSensor® Cell Lines use GeneBLAzer® technology to provide you with a reliable, rapid, and sensitive assessment of the intracellular status of therapeutically important signal transduction pathways upon treatment with drug candidates or other stimuli. Measure compound potency and selectivity, and analyze key pathways activated or down-regulated by compounds.

The SelectScreen™ Cellular Pathway Service is also available with the CellSensor® Cell Lines for rapidly screening or profiling your compounds in dozens of validated cellular assay.

Visit www.invitrogen.com/cellsensor to view the current pathways available.

Stealth™ RNAi collections—designed for screening success

The Stealth™ RNAi Human Kinase Collection is the first chemically modified RNAi collection for rapid, high-throughput functional screening of human kinases. This collection provides the distinct advantages of the most up-to-date bioinformatics, combined with the enhanced specificity and high hit rate of Stealth™ RNAi, all in a convenient, transfection-ready format.

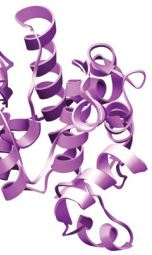
The Stealth™ RNAi Human Kinase Collection contains 636 human kinase targets, with three individual nonoverlapping Stealth™ RNAi duplexes per target (1,272 duplexes). Each duplex is provided at 2 nmol per well, which is sufficient to perform ~200 transfections. The easy-to-use 96-well format is ideal for either manual or automated screening. Positive and negative controls and blank wells for user-designed controls are conveniently included on each 96-well plate.

Ultimate™ ORF Clones

Ultimate™ ORF Clones are human and mouse open reading frames (ORFs) stringently qualified and designed to provide you with ultimate utility for all your downstream application needs. To guarantee high-quality, reliable clones, Ultimate™ ORFs are full-insert sequenced. In addition, before shipping, the clone culture must pass a stringent QC test that authenticates the identity of the clone.

No matter what your downstream application needs, the Ultimate™ ORFs are ready to go. All Ultimate™ ORFs are provided in a Gateway® entry vector. This means you can rapidly and efficiently recombine the ORF of interest into any expression vector and go straight to gene analysis in your system of choice. Simply choose from Invitrogen's comprehensive selection of Gateway®-compatible expression vectors (destination vectors), or convert your vector of interest into a destination vector using the Gateway® Vector Conversion Kit.

All Ultimate™ ORF Clones can be readily identified and ordered via an online search using the Ultimate™ ORF Browser at www.invitrogen.com/clones.



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Invitrogen's ProtoArray® Kinase Substrate Profiling Service will catapult your research into pathway elucidation by screening your kinase of interest on a high-density protein microarray with thousands of protein substrates. In just six weeks, we can identify novel, biologically relevant kinase substrates. Choose from our Human ProtoArray® with approximately 8,000 proteins, or Yeast ProtoArray® with over 4,000 proteins.

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Continue your partnership with our custom biochemical and cell-based assay development services. Count on professionalism, commitment to quality, and fast turnaround to get the results you need quickly (Table 6).

Table 6—Technology options for your custom assay solution.

Assay platform	Detection technology	Description/advantages
Cell-based assay technologies		
LanthaScreen™ GFP Cellular Assays	TR-FRET	Study posttranslational modifications of physiological substrates in a live-cell context
GeneBLAzer® cell-based assay system using beta-lactamase (*bla*) technology	Fluorescence resonance energy transfer (FRET)	Membrane-permeant FRET-based substrate provides a ratiometric, miniaturizable, nontoxic cell-based assay for therapeutically important target
Voltage Sensor Probes (VSP) for ion channel screening		Can be applied to any target that changes the membrane potential. Ideally suited for biological screening and rapid (sub-second time scale) membrane potential changes
Green fluorescent protein (GFP)	Fluorescence intensity (FI)	Nonradioactive detection and localization of drug targets in live cells
Lumio™ site-specific fluorescent labeling		
Biochemical assay technologies		
Z′-LYTE® FRET kinase assays	FRET	Universal platform with short assay development time. Real-time ratiometric detection eliminates well-to-well variations
Adapta™ TR-FRET assays	TR-FRET	Universal ADP detection assay
LanthaScreen™ TR-FRET assays		Minimizes background fluorescence by utilizing a variety of acceptor molecules
PolarScreen™ FP-based assays	Fluorescence polarization (FP)	Homogeneous and versatile

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