HUMAN HEALTH

ENVIRONMENTAL HEALTH

DISCOVER MORE

TR-FRET SOLUTIONS

TR-FRET Technology Solutions

Reagents, Detection, Automation and Services

PerkinElmer®

For the Better

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DISCOVER MORE TR-FRET SOLUTIONS TO ACCELERATE RESEARCH

With PerkinElmer and TR-FRET technology, you always get more – more experience, more solutions, more support. PerkinElmer's TR-FRET delivers a vast range of targets with greater precision to help you accelerate the development and optimization of sensitive assays for your research.

TR-FRET technology is part of PerkinElmer's Complete Solution including reagents, instruments, automation and services.

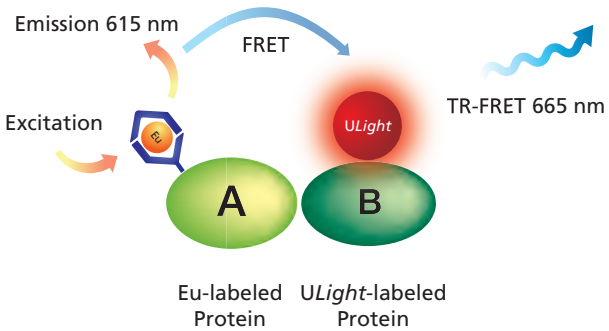
A history of innovation

The first company to commercialize europium-based TR-FRET, PerkinElmer is actively evolving TR-FRET's performance and applications with innovations such as LANCE *Ultra* cAMP and applications in epigenetics.

PerkinElmer has over 30 years' experience in defining, synthesizing and optimizing chelate and chelate-based bioanalytical assays (for both research assays and diagnostic assays).

A clearer signal

A homogeneous proximity assay technology, TR-FRET combines the advantages of TR (Time-Resolution) with FRET (Fluorescence Resonance Energy Transfer) principles using energy donor and energy acceptor fluorophore labels. Upon excitation, the donor fluorophore donates its emission energy to the acceptor fluorophore when the molecules are in close enough proximity. Once the energy is accepted, the acceptor fluorophore emits at a different emission wavelength, which can be measured to assess the formation of the biological complex.



- TR-FRET offers a long donor fluorescence lifetime
- This allows decay of any short-lived emissions
- Background interferences are thereby eliminated
- This results in a clean energy transfer signal

A choice of platforms

PerkinElmer offers two TR-FRET platforms: the original LANCE (Lanthanide Chelate Excite) and the next-generation LANCE *Ultra*. Both use europium (Eu) chelate donor dyes but have different acceptors. Europium permits time-delayed signal detection, virtually eliminating background interference. As an acceptor dye, a small, red-shifted U*Light*™ is used in LANCE *Ultra* assays. U*Light* provides a strong signal with minimal steric hindrance that is perfect for kinase and GPCR applications. LANCE uses a large allophycocyanin (APC) acceptor dye well suited for indirect assays. Every PerkinElmer TR-FRET assay is developed on the PerkinElmer EnVision Multilabel Plate Reader to ensure consistent and reproducible results.

ENHANCE YOUR RESEARCH WITH ENLIGHTENING TECHNOLOGY

PerkinElmer's LANCE TR-FRET signal detection is based on a combination of a europium chelate donor dye with your choice of a large or small acceptor dye.

LANCE europium chelates

LANCE europium chelates have been optimized for assay performance, signal level, energy transfer efficiency, background and sensitivity of the assay to interferences. The results are a superior S:B ratio and a very sensitive TR-FRET assay. You use less label and get more efficient use of substrate. With PerkinElmer's LANCE TR-FRET, you get:

- **Biocompatibility:** LANCE europium chelates and labeling reagents provide mild and controlled coupling reactions that maintain biological function without increasing hydrophobicity, resulting in minimized backgrounds.
- **Stability:** LANCE europium chelates work efficiently under the conditions required for typical bioassays to be performed, which may include addition of divalent cations and stop reagents (in the case of kinase assays). No potassium fluoride (KF) is required for stability.
- **Signal:** LANCE europium chelates provide the high signal needed for sensitive assays with good intrachelate energy transfer (ligand-to-metal ET).
- **Low background:** LANCE europium chelates are designed for minimal background, further improving the signal-to-background ratio.

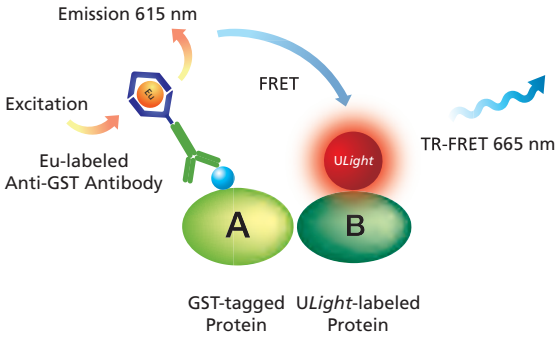
Acceptor dyes

Choose from two acceptor dyes: U*Light* dye or SureLight® allophycocyanin (APC). Both acceptor dyes have a red-shifted emission that is less sensitive to quenching by colored compounds, which eliminates the need for ratiometric analysis.

U*Light* acceptor dye

U*Light* is a small, bright dye developed for direct labeling of molecules of any size. The small size of the dye reduces steric hindrance to the labeled molecule, making it ideal for direct labeling of peptides, small molecules and enzymatic substrates.

- Small dye (<800 Da)
- Reduced steric hindrance to labeled molecule
- Ideal for direct coupling/direct labeling of molecules of any size, even small molecules and peptides
- Excellent for enzymatic assays using directly labeled substrates
- Minimal effect on solubility of labeled molecule, due to hydrophilic nature of dye



Upon excitation of the europium chelate at 320 or 340 nm, energy is transferred to the U*Light* acceptor dye, resulting in the emission of light at 665 nm. The intensity of light emission is proportional to the level of complexed molecules.

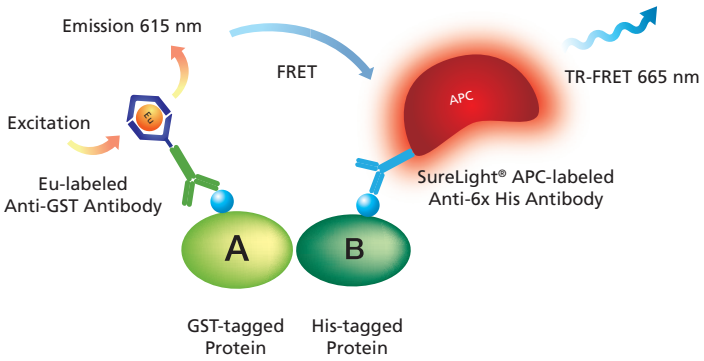
SureLight® APC acceptor dye

SureLight® APC is a larger dye derived from a light-harvesting protein. Its structure allows it to act as a large antenna, making it good for studying indirect assays that span wider distances.

- Large dye (>100,000 Da)
- Can act as large antenna
- Ideal for indirect assays that span wider distances
- Excellent for studying protein-protein interactions

Discover More Support

The Assay Support Knowledge (ASK) base contains detailed information on assay development, protocols, tips, FAQs, citations and troubleshooting. Accessible 24 hours a day, 7 days a week online at www.perkinelmer.com/ask. Or contact a technical support specialist by phone or e-mail for more scientific support.



In LANCE assays, the energy is transferred to the APC acceptor dye molecule, which in turn emits light at 665 nm.

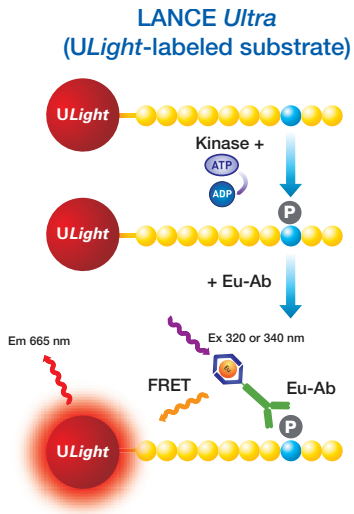
INCREASE YOUR RANGE OF KINASE TARGETS

Kinases are an important target class in understanding cell signaling and in drug development.

The widest portfolio

We have tested and validated over 300 commercially available kinases in LANCE *Ultra* kinase assay format. We provide confidence through data transparency by supplying:

- **Test conditions:** Rapid assay setup to move straight into optimized assay performance.
- **S:B data:** Confidence in proven performance of the kinase on specific substrates; no guessing.
- **Phospho-motifs:** Substrate sequences are provided.
- **Assay options:** Multiple substrates are tested so you can select the best peptide sequence and S:B for your kinase.



In kinase assays, the binding of a Eu-labeled anti-phospho-substrate antibody to the phosphorylated U*Light*-labeled substrate brings donor and acceptor molecules into close proximity. After irradiation of the kinase reaction at 320 or 340 nm, the energy from the Eu donor is transferred to the U*Light* acceptor dye, which in turn generates light at 665 nm. The intensity of the light emission is proportional to the level of U*Light*-substrate phosphorylation.

Minimize variables with our two-component assay

LANCE *Ultra* is a two-component assay that combines direct labeling of the kinase substrate with U*Light*, an innovative emission dye for optimum binding efficiency, reducing steps and assay prep time.

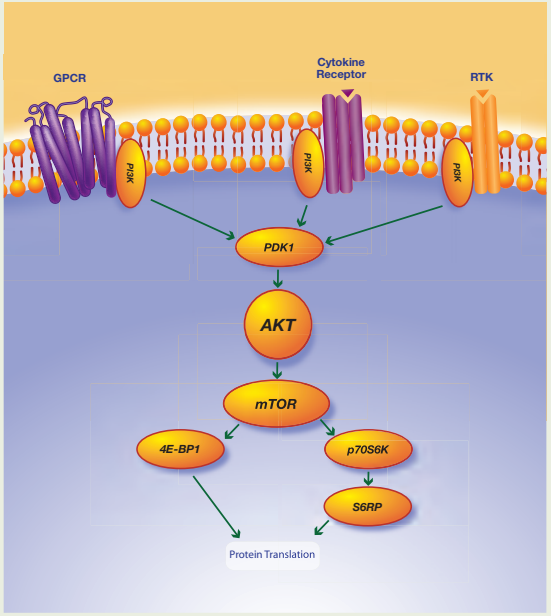
Build your own LANCE TR-FRET kinase assay

We have a broad selection of LANCE TR-FRET donor and acceptor dye-labeled reagent pairs. Take your tagged recognition sequence and combine with our europium-labeled anti-phospho-antibody and either SureLight® allophycocyanin-streptavidin or U*Light*-streptavidin. We can also custom label your proprietary peptide sequences with our U*Light* dye. For more information on our custom labeling services, please refer to page 10.

Discover More Transparency

PerkinElmer has a history of data transparency in TR-FRET, providing data preferences to its customers. With pre-tested kinase and substrate pairs data provided, customers have an advanced start on their research. Visit www.perkinelmer.com/kinaseselector to see how easy it is to find the right assay for your kinase.

mTOR is a serine/threonine kinase that regulates cell growth and cell proliferation. The mTOR pathway is dysregulated in human diseases, including certain cancers. mTOR has been shown to phosphorylate p70S6K. This event stimulates initiation of protein synthesis via activation of S6 ribosomal protein and other proteins involved in translation. mTOR has also been shown to phosphorylate residues of 4E-BP1. Upon phosphorylation by mTOR, 4E-BP1 dissociates from eIF4E, allowing eIF4E to activate mRNA translation.



Phosphorylation of both p70S6K and 4E-BP1 by mTOR has been demonstrated using LANCE *Ultra* kinase assays. In Figure 1, U*Light*-p70S6K peptide substrate comprised of the amino acids including and surrounding the critical Thr389 residue was used in conjunction with europium-labeled anti-phospho p70S6K (Thr389) antibody. The IC_{50} for inhibition of mTOR kinase by wortmannin is shown.

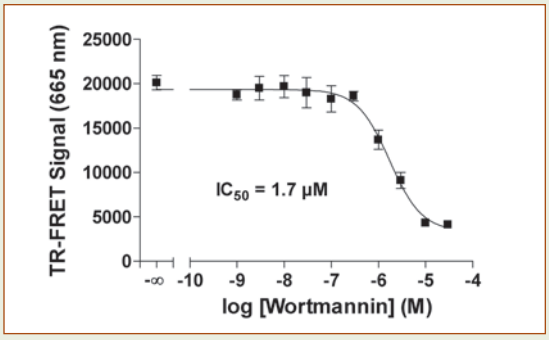


Fig. 1. Inhibition of mTOR kinase phosphorylation of the U*Light*-p70S6K peptide by wortmannin.

In a second experiment, U*Light*-4E-BP1 peptide substrate comprised of the amino acids including and surrounding the critical Thr37 and Thr46 residues was used in conjunction with europium-labeled anti-phospho 4E-BP1 (Thr37/46) antibody. Figure 2 shows inhibition of mTOR kinase by PI-103, with determination of IC_{50}.

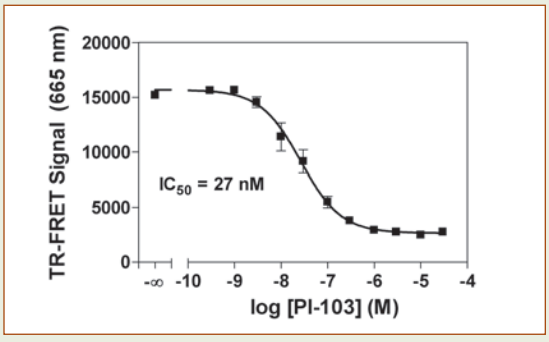


Fig. 2. Inhibition of mTOR kinase by PI-103.

The ability to utilize peptides derived from biologically relevant substrates (for example, p70S6K and 4E-BP1) in the LANCE *Ultra* kinase assay provides a key benefit in studying mTOR and other kinases. Core peptide sequences are disclosed for all of our U*Light* substrates. Our kinase selector tool provides further validation data on substrate selection and assay performance. Together, these provide the tools you need to be confident you are running the best assay for your kinase.

For further details, refer to LANCE Ultra *Technical Notes #26 and #29 available at www.perkinelmer.com/lance.*

UNMATCHED SENSITIVITY FOR ALL YOUR GPCR TARGETS

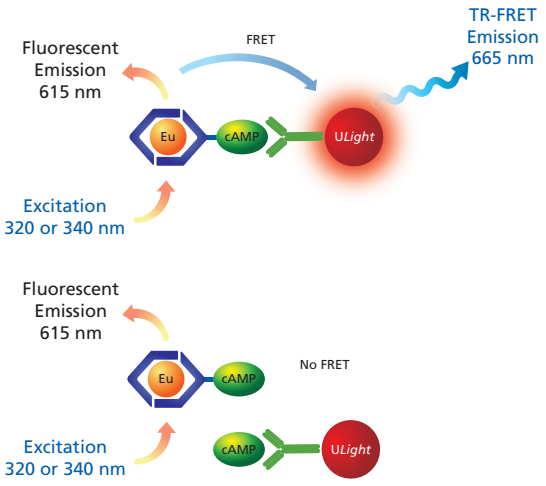
There is an increased demand for more robust and highly sensitive methods of measuring responses to GPCRs. Their activity can be assessed by measuring cyclic AMP (cAMP), an intracellular second messenger released upon stimulation by appropriate agonists.

LANCE *Ultra* cAMP

LANCE *Ultra* cAMP offers the widest assay window and dynamic range, providing more accurate results even when miniaturizing protocols for the most "difficult to screen" targets, including G_i-coupled GPCR antagonist assays. LANCE *Ultra* cAMP provides:

- Unmatched assay sensitivity and signal stability – even with overnight incubation
- Optimized screening of difficult targets and G_i antagonists
- Easier detection of cAMP response from endogenous receptors – use fewer cells per well
- Robust sensitivity when miniaturized to 1536-well format for uHTS
- Trusted results: stable pharmacology over time with consistent rank order potencies
- Reproducible results with the highest Z' values

LANCE *Ultra* cAMP Assay Principle



In the absence of free cAMP (top panel), maximal TR-FRET signal is achieved. Free cAMP produced by stimulated cells competes with the Eu-cAMP tracer for binding to the U*Light* monoclonal antibody (bottom panel), causing a decrease in TR-FRET signal proportional to the concentration of cAMP produced.

LANCE cAMP

Our first-generation LANCE cAMP kit continues to provide outstanding performance in a three-component assay, combining homogeneous TR-FRET technology for excellent excitation/emission discrimination. GPCR cAMP assays. Start with the best: LANCE.

Discover More Cells

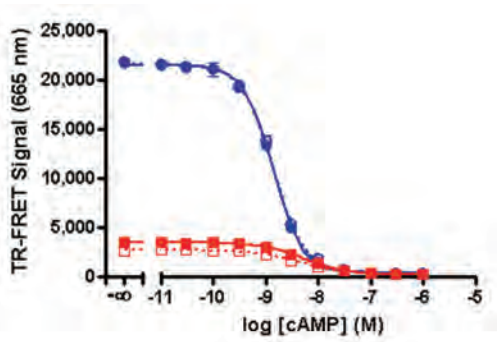
Discover how simple it can be to run cAMP assays. With cAMPZen® ready-to-use frozen cells, you can eliminate the hassle of cell culture with a functionally tested GPCR transfected cell line already validated for LANCE cAMP assays. Please visit www.perkinelmer.com/gpcr for more information.

Case Study: 5-HT$_{1A}$ Serotonin Receptor GPCR

The 5-HT$_{1A}$ serotonin receptor is part of a class of GPCRs found in the central and peripheral nervous systems. As a G_i-coupled GPCR, the 5-HT$_{1A}$ target faces the challenges associated with obtaining a suitable assay window, especially when working with antagonists. Because of its superior assay window and outstanding performance, the LANCE *Ultra* cAMP kit is ideal for studying any GPCR – even when working in 1536-well format.

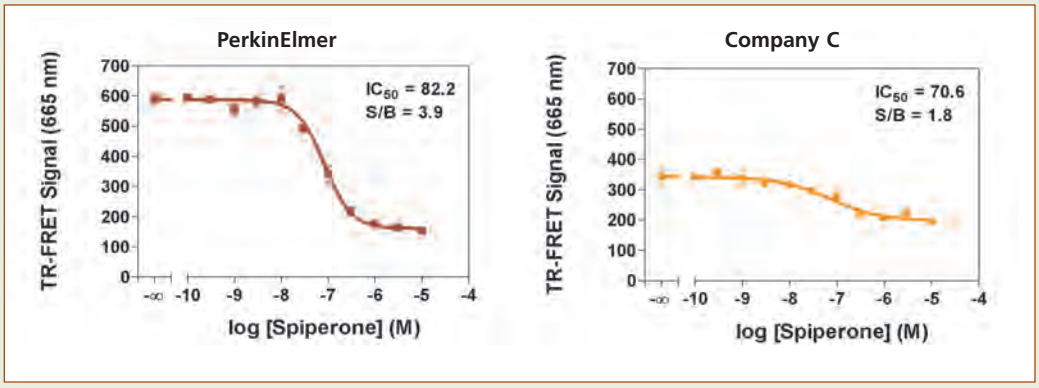
Based on lower IC_{50} values derived from cAMP standard curves and forskolin concentration-response curves, PerkinElmer's new LANCE *Ultra* cAMP assay was shown to be more sensitive than comparative kits. In 1536-well screening on the EnVision Multilabel Plate Reader, the LANCE *Ultra* assay showed stable Z' factor values even after overnight incubation (Z'=0.64-0.71). In contrast, the comparative TR-FRET kit showed lower Z' factor values at all reading times.

This enhanced sensitivity allows the use of fewer cells per well in miniaturized and automated applications with no need to change protocol or re-engineer cells.



	S/B	IC_{50} (nM)
● LANCE *Ultra* (1 hr)	69	1.3
○ LANCE *Ultra* (O/N)	70	1.4
■ Company C (1 hr)	17	5.4
□ Company C (O/N)	13	4.6

cAMP standard curve. LANCE *Ultra* cAMP kit allows detection of the smallest changes in cAMP while providing an assay with superior S:B ratio compared to other TR-FRET kits; IC_{50} values were stable following overnight incubation.



Spiperone [5-hydroxytryptamine] antagonist response curves in 1536-well plate format shows 2.2-fold greater signal window with the LANCE *Ultra* cAMP assay (left figure) compared to Company C (right figure).

For more information on study design and results, download our Application Note: "Measuring Performance and Sensitivity of an Automated and Miniaturized LANCE *Ultra* cAMP Assay for the G_i-coupled 5-HT$_{1A}$ Receptor – a Comparative Study." Or visit www.perkinelmer.com/openthewindow.

TOOLS TO BUILD YOUR RESEARCH

The versatility of LANCE TR-FRET technology allows assembly of donor and acceptor dye pairs for use in protein binding assays. Choose your preferred donor – and acceptor-labeled reagents to design your own assay. This is assay design at its most flexible, featuring the most common affinity biomolecules and europium chelate-labeling reagents.

Toolbox reagents

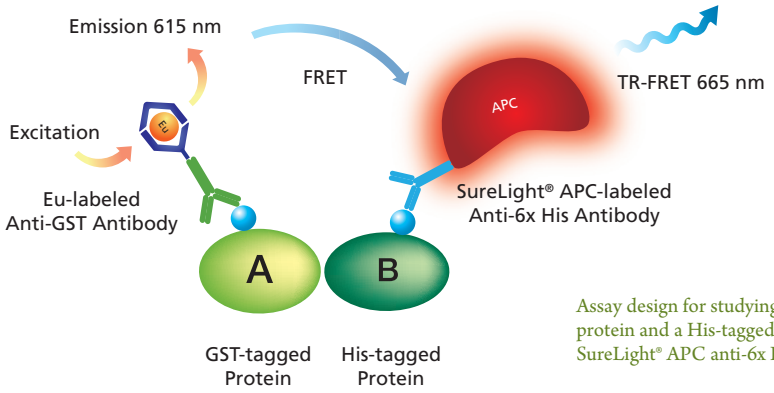
PerkinElmer offers a range of LANCE toolbox reagents that can be used to develop any assay, no matter what you want to study. Design your assay using our donor and acceptor fluorophore-labeled streptavidin, anti-fusion tag antibodies, anti-species antibodies, protein A and protein G reagents, biotin or WGA.

Discover More Applications

A wide variety of applications has been studied using LANCE TR-FRET technology:

- Kinases
- GPCR activation
- Protein-protein binding
- Protein-nucleic acid binding
- Protein-small molecule binding
- Receptor-ligand binding
- Nuclear receptors
- Receptor dimerization
- Ubiquitination/post-translational modifications
- Biomarker assays
- Proteases
- Other enzymatic assays

For more information on each, please see reference section publications on page 20.



Assay design for studying the interaction between a GST-tagged protein and a His-tagged protein, using LANCE Eu-anti-GST and SureLight® APC anti-6x His toolbox reagents.

LANCE Toolbox Reagents

Donor fluorophore reagents

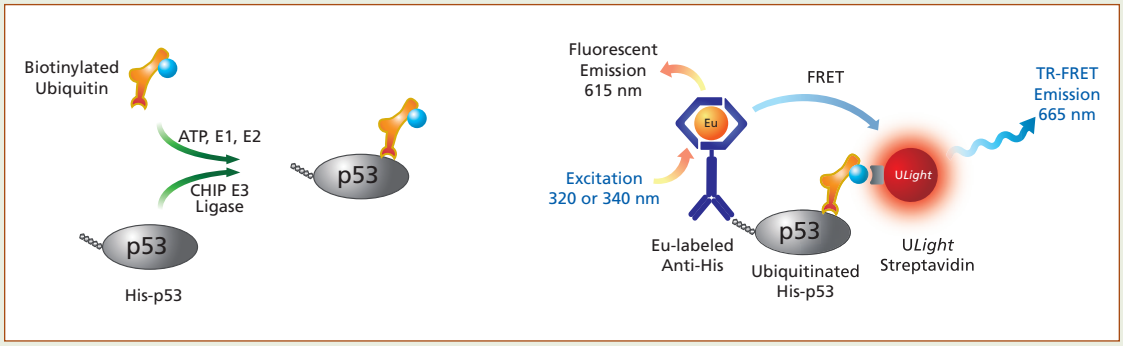
- Eu-anti-6x His
- Eu-anti-GST
- Eu-anti-HA
- Eu-anti-c-myc
- Eu-protein G
- Eu-streptavidin
- Eu-biotin
- Eu-anti-human IgG
- Eu-anti-mouse IgG
- Eu-anti-rabbit IgG

Acceptor fluorophore reagents

- U*Light* and SureLight® APC streptavidin
- U*Light* and SureLight® APC anti-6x His
- U*Light* and SureLight® APC anti-GST
- SureLight® APC anti-c-myc
- SureLight® APC anti-FLAG
- SureLight® APC anti-mouse
- SureLight® APC anti-rabbit
- U*Light* protein A
- SureLight® APC WGA

We also carry a variety of europium labeling reagents, if you would prefer to directly label your own biomolecule.

Protein ubiquitination is an important regulatory mechanism in many cellular processes, including cell cycle progression, antigen presentation, apoptosis, signal transduction, transcriptional activation and endocytosis. A LANCE *Ultra* assay was developed for the detection of p53 ubiquitination. In this assay, biotinylated ubiquitin molecules are ligated to a His-tagged p53 by E1, E2 and E3 ubiquitination enzymes. p53 ubiquitination is then detected using the TR-FRET reagents U*Light*-streptavidin and europium-labeled anti-6x His antibody.



LANCE *Ultra* p53-CHIP ubiquitination assay.

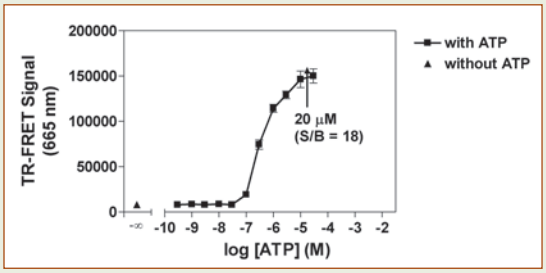
Assay Protocol:

A) Ubiquitination reaction (volume of 10 µL):

1. Transfer 5 µL of 2X Enzyme Mix (E1, UbcH5a, CHIP) to the well.
2. Add 2.5 µL of 4X Substrate Mix (p53-His, biotin-ubiquitin).
3. Add 2.5 µL of 4X ATP stock solution.
4. Incubate at 23°C for 90 min.

B) Detection reaction (final assay volume of 20 µL):

1. Add 5 µL of the EDTA 4X Stop solution to the enzymatic reaction.
2. Incubate at 23°C for 5 min.
3. Add 5 µL of 4X Detection Mix (U*Light*-SA, Eu-anti-6x His antibody).
4. Incubate at 23°C for 60 min.
5. Read on the EnVision Multilabel Plate Reader in TR-FRET mode.



ATP titration. ATP was titrated from 300 pM to 100 µM in the enzymatic reaction. The assay was performed using the following final concentrations in the well: 2 nM E1, 100 nM E2, 20 nM E3, 50 nM p53-His, 200 nM bio-ubiquitin 10 mM EDTA, 2 nM Eu-anti-6x His antibody, 100 nM U*Light*-SA.

Results demonstrate the use of LANCE toolbox reagents in the development of a sensitive and robust assay for measuring *in vitro* ubiquitination of p53. We offer a broad range of LANCE toolbox reagents to facilitate the development of your TR-FRET assay.

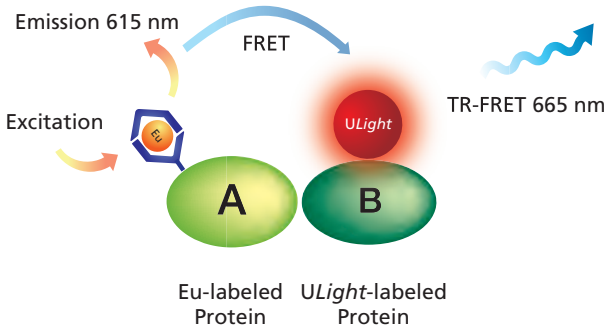
For more information on our assay development services, please visit www.perkinelmer.com/onpoint.

CUSTOM TR-FRET SOLUTIONS

You can rely on the expertise of PerkinElmer manufacturing scientists to deliver quality and purity based on knowledge and experience. Let our team of experts perform your custom labeling requirements, leaving you the time to focus on assay performance.

Custom labeling

Choose from custom labeling your biomolecule with LANCE europium, U*Light* dye or APC. Many assays can benefit from direct labeling due to the FRET proximity requirement. Our small europium chelates (~700-1000 Da) and U*Light* dye (<800 Da) allow for direct labeling with minimal added steric hindrance to your biomolecule.



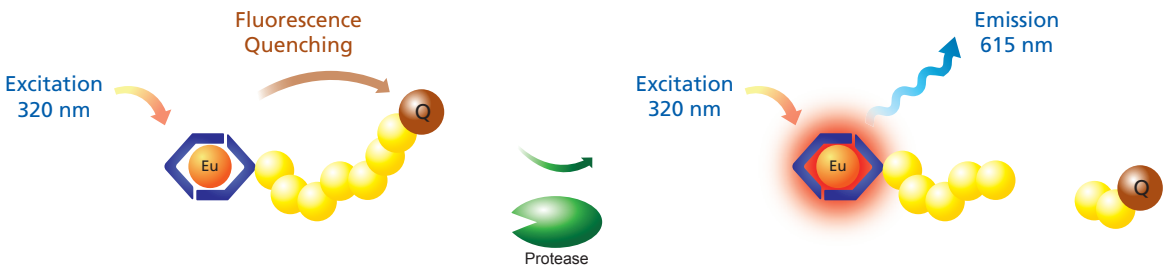
Using two directly labeled proteins in a protein-protein interaction can result in higher signal-to-background and fewer addition steps.

OnPoint Reagent Services

Let PerkinElmer's OnPoint℠ Reagent Services develop an assay to your specifications. The OnPoint team can recommend solutions from one of the industry's broadest portfolios of reliable and innovative technologies. For more information on our custom labeling and assay development services, please visit www.perkinelmer.com/onpoint.

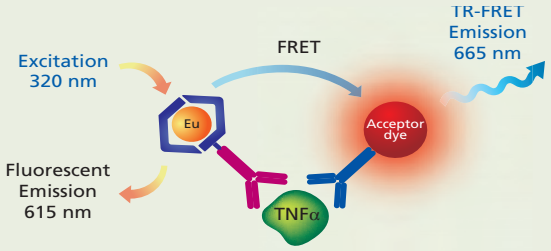
TruPoint™ custom assays

Prefer a fluorescence quench assay? TruPoint technology relies on quenching of the europium lanthanide fluorescence by an organic acceptor dye. The separation of the quencher from the chelate results in a signal increase enabling sensitive homogeneous assays. TruPoint assays are utilized especially for measuring separation assays, catalyzed, for example, by proteases.



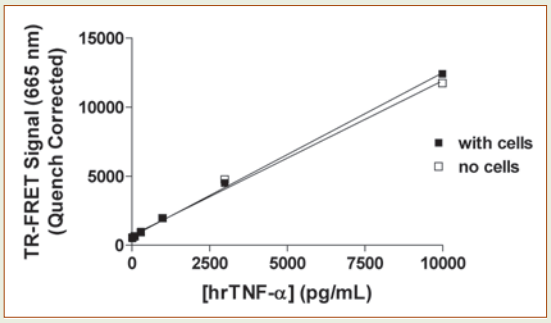
TNFα is a pro-inflammatory cytokine produced primarily by activated monocytes, macrophages and phagocytic cells. It is implicated in both acute and chronic inflammation. TNFα is selectively cytotoxic for many transformed cells *in vitro* and *in vivo*, leading to necrosis.

A LANCE assay was developed for the detection of TNFα secreted by THP-1 cells. In the sandwiching immunoassay, one anti-TNFα antibody is labeled with donor fluorophore Eu chelate, and a second anti-TNFα antibody is labeled with an acceptor fluorophore.



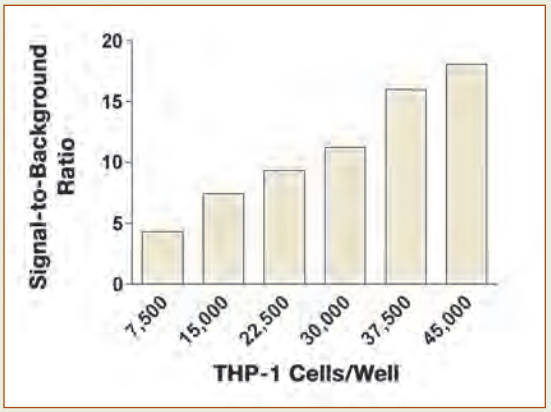
LANCE sandwiching immunoassay for TNFα detection.

Calibrator curves for human recombinant TNFα were made in the presence or absence of 30,000 non-differentiated THP-1 cells. The recombinant cytokine and cells were dispensed in culture medium in a volume of 30 µL in a 384-well assay format. The antibodies were added to the reaction mix in 10 µL. Signal was measured after 2-hour incubation.



Calibration curve for TNFα.

THP-1 cells are derived from the peripheral blood of a patient with human acute monocytic leukemia. These cells become phagocytic and secrete TNFα upon differentiation with phorbol ester and vitamin D3. LANCE TNFα assays were performed with different concentrations of THP-1 cells stimulated with LPS in 384-well assay format. Signal-to-background (S:B) ratios represent the signal ratio between LPS-stimulated and non-stimulated cell samples.



Detection of TNFα produced in stimulated cells plated at different densities.

For this sandwich immunoassay assay, directly labeled antibodies were used to develop a TR-FRET detection assay for TNFα. Direct labeling can be performed using LANCE europium labeling reagents, or can be requested through OnPoint custom services. Labeling reagents and custom services provide you with the flexibility to take advantage of TR-FRET-based assays for your analyte of interest.

For more information, refer to the poster: "New LANCE Assays for the High Throughput Quantitation of Cytokine Biomarkers," available at www.perkinelmer.com/postergallery.

OPTIMIZE RESEARCH WITH INSTRUMENTAL TECHNOLOGY

PerkinElmer is the detection expert! Offering a total solution for your assay needs, we carry a variety of highly sensitive instruments and superior microplates to use for your TR-FRET assay.

Choose high-quality microplates built by the instrument and assay experts at PerkinElmer.

PerkinElmer Microplates

Trust PerkinElmer for high-quality microplates that deliver better performance and better results.

- Plates optimized for fluorescence measurements
- 96-, 384- and 1536-well formats
- Validated for LANCE TR-FRET applications

ViewLux™ Ultra HTS Microplate Imager

The ViewLux instrument is an ultra-high throughput microplate imager for multilabel detection. Image and analyze an entire microplate all at once.

- Cooled CCD camera and a special telecentric lens image and analyze a 1536-well plate in less than 30 seconds
- Supports all plate types and densities, up to and beyond 1536
- Supports both robot-loading and batch mode operation – up to 65 plates can be loaded for unattended operation

Run and miniaturize TR-FRET assays to 1536-well format easily with ViewLux.

Access the latest in best-in-class detection with VICTOR.

VICTOR *X* Multilabel Plate Reader

VICTOR instruments deliver unsurpassed flexibility, speed and performance to research labs of all sizes.

- Performs the work of a fluorometer, luminometer, time-resolved fluorometer and photometer
- Features multilabel, multitask counters for all light-emitting and light-absorbing detection technologies
- Operates as a stand-alone instrument or integrates into a robotic system

EnVision Multilabel Plate Reader

This state-of-the-art dual-detector model is among the fastest HTS readers in the market.

- Compatible with kinetic measurements, enzyme assays and other cell-based assays
- Includes two detectors, enabling simultaneous dual-wavelength reading: top and bottom reading, filter-based measurements, as well as monochromator readings are possible
- Fully field upgradable with stackers, temp control, dispenser, ultrasensitive luminescence, TRF-laser and Alpha Technology

Get the sensitivity of a filter-based system and the flexibility of a monochromator in one system with EnVision.

Automate TR-FRET reagent addition to any plate format.

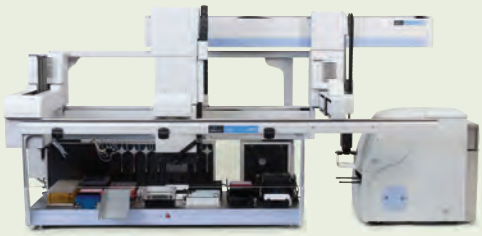
JANUS Automated Workstation

Easy to use and flexible to support a wide array of applications, JANUS delivers the benefits of automation to every size lab.

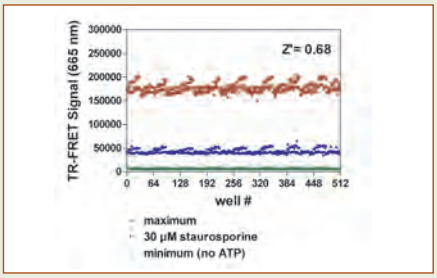
- Improves your lab's overall efficiency with walk-away automation
- Enhances dispensing precision and accuracy
- Increases throughput from 96- to 1536-well plates
- Maintains assay sensitivity and reproducibility
- Minimizes errors in multi-user laboratories
- Supports your choice of reagents, assays and kits

Case Study: LANCE *Ultra* Kinase Assay on JANUS/EnVision

Automation of assays can help reduce user error and assay variability, as well as provide a way to easily miniaturize your TR-FRET assays and reduce cost. Shown is the automation and miniaturization of an IκKβ LANCE *Ultra* kinase assay, demonstrating LANCE *Ultra* technology in combination with the JANUS Modular Dispense Technology Automated Workstation. These data demonstrate that the LANCE *Ultra* platform, combined with the JANUS Automated Workstation with Modular Dispense Technology, is capable of automation and substantial miniaturization of Ser/Thr kinase assays. Moreover, the assays have the potential for reduction of reagents, without compromising assay quality. For more information, refer to the poster: "Development, Automation and Miniaturization of High Throughput Serine/Threonine Kinase Assays Using the LANCE *Ultra* Platform," available at www.perkinelmer.com/postergallery.



Integration of the JANUS with the EnVision Multilabel Plate Reader.



Intra-plate variability of automated IκKβ kinase assay in 1536-well format.

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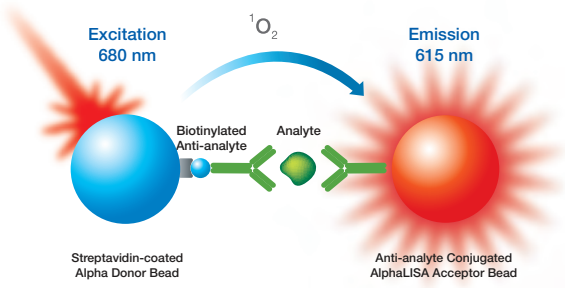
When a biological reaction brings Alpha Donor and Acceptor beads into close proximity, conditions are set to allow a cascade of chemical reactions leading to a greatly amplified signal that contributes to detection sensitivity down to the attomole level.



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AlphaScreen® *SureFire*® assays are the perfect complement to your LANCE *Ultra* kinase assay. These kits enable the highly sensitive and precise interrogation of various signaling pathways, receptors and kinase targets, and the measurement of full-length, endogenous protein phosphorylation in a cell-based format. AlphaScreen® *SureFire*® makes it possible to culture cells and detect key analytes using an "all-in-one-well" format. Alternatively, cells can be used in multiple assays analyzing pathways or allowing for kinase profiling. This eliminates time-consuming separation and wash steps. Measure cellular kinase assays using one of our AlphaScreen® *SureFire*® kits.

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ORDERING INFORMATION

LANCE GPCR kits

Product	Size	Catalog Number
LANCE *Ultra* cAMP kit	1,000 assays (in 384-well format) 10,000 assays (in 384-well format) 50,000 assays (in 384-well format)	TRF0262 TRF0263 TRF0264
LANCE cAMP kit	500 assays (in 384-well format) 1,000 assays (in 384-well format) 10,000 assays (in 384-well format) 50,000 assays (in 384-well format)	AD0262 AD0262E AD0263 AD0264

LANCE buffers and solutions

Product	Size	Catalog Number
10X LANCE detection buffer (*not* for use in LANCE cAMP assays)	250 mL	CR97-100
7.5% BSA solution	50 mL	CR84-100

LANCE *Ultra* kinase substrates and antibody pairs

Substrate	Substrate + Matching Antibody	Size (for 20 µL reaction)	Catalog Number	Phospho-Motif
U*Light*-CREBtide	U*Light*-CREBtide (Ser133)	1,000 assay points 10,000 assay points	TRF0107-D TRF0107-M	RRP**S**YRK
	Europium-anti-phospho-CREB (Ser133)	1,562 assay points 15,625 assay points	TRF0200-D TRF0200-M	
U*Light*-MBP	U*Light*-Myelin Basic Protein Peptide	1,000 assay points 10,000 assay points	TRF0109-D TRF0109-M	VTPR**T**PPP
	Europium-anti-phospho-Myelin Basic Protein	1,562 assay points 15,625 assay points	TRF0201-D TRF0201-M	
U*Light*-Crosstide	U*Light*-Crosstide (GSK-3α Ser21)	1,000 assay points 10,000 assay points	TRF0106-D TRF0106-M	PRTS**S**FAE
	Europium-anti-phospho-Crosstide (GSK-3α Ser21)	1,562 assay points 15,625 assay points	TRF0202-D TRF0202-M	
U*Light*-PLK	U*Light*-PLK (Ser137) Peptide	1,000 assay points 10,000 assay points	TRF0110-D TRF0110-M	RRR**S**LLE
	Europium-anti-phospho-PLK (Ser137)	1,562 assay points 15,625 assay points	TRF0203-D TRF0203-M	
U*Light*-PKC	U*Light*-PKC Substrate (Ala25Ser)	1,000 assay points 10,000 assay points	TRF0108-D TRF0108-M	RKG**S**LRQ
	Europium-anti-phospho-PKC Peptide (Ala25Ser)	1,562 assay points 15,625 assay points	TRF0207-D TRF0207-M	
U*Light*-IκBα	U*Light*-IκB-α (Ser32/36) Peptide	1,000 assay points 10,000 assay points	TRF0113-D TRF0113-M	RHD**S**GLD**S**M
	Europium-anti-phospho-IκB-α (Ser32/36)	1,562 assay points 15,625 assay points	TRF0206-D TRF0206-M	
U*Light*-Histone H3 (Thr3/Ser10)	U*Light*-Histone H3 (Thr3/Ser10) Peptide	1,000 assay points 10,000 assay points	TRF0125-D TRF0125-M	AR**T**KQTARK**S**TG
	Europium-anti-phospho-Histone H3 (Ser10)	1,562 assay points 15,625 assay points	TRF0210-D TRF0210-M	
	Europium-anti-phospho-Histone H3 (Thr3)	1,562 assay points 15,625 assay points	TRF0211-D TRF0211-M	
U*Light*-mTOR	U*Light*-mTOR (Ser2448) Peptide	1,000 assay points 10,000 assay points	TRF0119-D TRF0119-M	TRTD**S**YSAG
	Europium-anti phospho-mTOR (Ser2448)	1,562 assay points 15,625 assay points	TRF0209-D TRF0209-M	

ORDERING INFORMATION

LANCE *Ultra* kinase substrates and antibody pairs (continued)

Substrate	Substrate + Matching Antibody	Size (for 20 µL reaction)	Catalog Number	Phospho-Motif
U*Light*-Glycogen Synthase	U*Light*-Glycogen Synthase (Ser641/pSer657) Peptide	1,000 assay points 10,000 assay points	TRF0131-D TRF0131-M	PA**S**VPPSPSLSRHSSPHQ(pS)ED
	Europium-anti-phospho-Glycogen Synthase (Ser641)	1,562 assay points 15,625 assay points	TRF0220-D TRF0220-M	
U*Light*-p70S6K	U*Light*-p70S6K (Thr389) Peptide	1,000 assay points 10,000 assay points	TRF0126-D TRF0126-M	FLGF**T**YVAP
	Europium-anti-phospho-p70S6K (Thr389)	1,562 assay points 15,625 assay points	TRF0214-D TRF0214-M	
U*Light*-Acetyl-CoA Carboxylase	U*Light*-Acetyl-CoA Carboxylase (Ser79) SAMS Peptide	1,000 assay points 10,000 assay points	TRF0118-D TRF0118-M	RSAM**S**GLHL
	Europium-anti-phospho-Acetyl-CoA Carboxylase (Ser79)	1,562 assay points 15,625 assay points	TRF0208-D TRF0208-M	
U*Light*-Cdc25C	U*Light*-Cdc25C (Ser216) Peptide	1,000 assay points 10,000 assay points	TRF0123-D TRF0123-M	YRSP**S**MPEN
	Europium-anti-phospho-(Ser) 14-3-3 Binding Motif, mAb 4E2	1,562 assay points	AD0192	
U*Light*-4E-BP1	U*Light*-eIF4E-binding Protein 1 (Thr37/46) Peptide	1,000 assay points 10,000 assay points	TRF0128-D TRF0128-M	ST**T**PGGTLFST**T**PG
	Europium-anti-phospho-eIF4E-binding Protein 1 (Thr37/46)	1,562 assay points 15,625 assay points	TRF0216-D TRF0216-M	
U*Light*-rpS6	U*Light*-40S Ribosomal Protein S6 (pSer235/Ser236) Peptide	1,000 assay points 10,000 assay points	TRF0129-D TRF0129-M	RRL(pS)**S**SLRA
	Europium-anti-phospho-40S Ribosomal Protein S6 (Ser235/236)	1,562 assay points 15,625 assay points	TRF0217-D TRF0217-M	
U*Light*-Topo IIα	U*Light*-DNA Topoisomerase 2-α (Thr1342) Peptide	1,000 assay points 10,000 assay points	TRF0130-D TRF0130-M	DEK**T**DDE
	Europium-anti-phospho-DNA Topoisomerase 2-α (Thr1342)	1,562 assay points 15,625 assay points	TRF0218-D TRF0218-M	
U*Light*-poly GT	U*Light*-poly GT	1,000 assay points 10,000 assay points	TRF0100-D TRF0100-M	[E**Y**(4:1)]n
	Europium-anti-phosphotyrosine (PT66)	7,810 assay points 156,200 assay points	AD0068 AD0069	
U*Light*-poly GAT	U*Light*-poly GAT	1,000 assay points 10,000 assay points	TRF0101-D TRF0101-M	[EA**Y**(1:1:1)]n
	Europium-anti-phosphotyrosine (PT66)	7,810 assay points 156,200 assay points	AD0068 AD0069	
U*Light*-IRS-1	U*Light*-IRS-1 (Tyr983) Peptide	1,000 assay points 10,000 assay points	TRF0120-D TRF0120-M	SRGD**Y**MTMQ
	Europium-anti-phosphotyrosine (PT66)	7,810 assay points 156,200 assay points	AD0068 AD0069	
U*Light*-JAK-1	U*Light*-JAK-1 (Tyr1023) Peptide	1,000 assay points 10,000 assay points	TRF0121-D TRF0121-M	DKEY**Y**TVKD
	Europium-anti-phosphotyrosine (PT66)	7,810 assay points 156,200 assay points	AD0068 AD0069	
U*Light*-CDK1	U*Light*-CDK1 (Tyr15) Peptide	1,000 assay points 10,000 assay points	TRF0122-D TRF0122-M	GEGT**Y**VVY
	Europium-anti-phosphotyrosine (PT66)	7,810 assay points 156,200 assay points	AD0068 AD0069	
U*Light*-TK	U*Light*-TK Peptide	1,000 assay points 10,000 assay points	TRF0127-D TRF0127-M	**Y**
	Europium-anti-phosphotyrosine (PT66)	7,810 assay points 156,200 assay points	AD0068 AD0069	

ORDERING INFORMATION

LANCE kinase kits for substrate identification

Product	Size	Catalog Number
LANCE *Ultra* KinaSelect™ kit for Ser/Thr kinases	5 x 250 assays (in 384-well format)	TRF0300-C
LANCE *Ultra* KinaSelect kit for Tyr kinases	1,000 assays (in 384-well format)	TRF0301-D

LANCE donor anti-phospho antibodies

Product	Size	Catalog Number
Eu-anti-phosphotyrosine PT66	50 µg 1 mg	AD0068 AD0069
Eu-anti-phosphotyrosine P-Tyr-100	10 µg 50 µg 1 mg	AD0203 AD0161 AD0162
Eu-anti-phosphotyrosine PY20	50 µg 1 mg	AD0066 AD0067
Eu-anti-phosphothreonine	10 µg	AD0094
Eu-anti-phospho-threonine-proline	10 µg	AD0099
Eu-anti-phospho-serine/threonine antibody	10 µg	AD0176
Eu-anti-phospho-serine/threonine-phenylalanine antibody	10 µg	AD0178
Eu-anti-phospho-serine/threonine-proline antibody	10 µg	AD0180
Eu-anti-phospho-PKA substrate antibody	10 µg	AD0182
Eu-anti-phospho-AKT substrate antibody	10 µg	AD0184
Eu-anti-phospho-serine substrate antibody	10 µg	AD0186
Eu-anti-phospho-(Ser) PKC substrate antibody	10 µg	AD0188
Eu-anti-phospho-(Ser) 14-3-3 binding motif antibody	10 µg	AD0190
Eu-anti-phospho-(Ser) 14-3-3 binding motif 4E2 antibody	10 µg	AD0192
Europium anti-phospho-ATF-2	10 µg 100 µg	TRF0212-D TRF0212-M
Europium anti-phospho-p38α	10 µg 100 µg	TRF0219-D TRF0219-M
Europium anti-phospho-MEK1/2	10 µg 100 µg	TRF0213-D TRF0213-M

ORDERING INFORMATION

Toolbox donor fluorophore reagents

Product	Size	Catalog Number
LANCE Eu-anti-6x His antibody	10 µg 50 µg 1 mg	AD0205 AD0110 AD0111
LANCE Eu-anti-c-myc antibody	10 µg 50 µg 1 mg	AD0206 AD0114 AD0115
LANCE Eu-anti-HA antibody	50 µg 1 mg	AD0084 AD0085
LANCE Eu-anti-GST antibody	10 µg 50 µg 1 mg	AD0252 AD0253 AD0254
LANCE Eu protein G	10 µg 50 µg 1 mg	AD0211 AD0070 AD0071
LANCE Eu-anti-human IgG	10 µg 50 µg 1 mg	AD0212 AD0074 AD0075
LANCE Eu-anti-mouse IgG	50 µg 1 mg	AD0076 AD0077
LANCE Eu-anti-rabbit IgG	50 µg 1 mg	AD0082 AD0083
LANCE Eu-biotin	10 nmoles	CR91-100
LANCE Eu-W1024-streptavidin	50 µg 1 mg	AD0062 AD0063
LANCE Eu-W8044-streptavidin	50 µg 1 mg	AD0060 AD0061

Toolbox acceptor fluorophore reagents

Product	Size	Catalog Number
U*Light*-streptavidin	1 nmole 10 nmoles 100 nmoles	TRF0102-D TRF0102-M TRF0102-R
SureLight® APC-streptavidin (for kinase assays)	1 mg 50 mg	CR130-100 CR130-150
SureLight® APC-streptavidin (for binding assays)	1 mg 30 mg	AD0201 AD0202
U*Light* anti-His antibody	1 nmole 10 nmoles 100 nmoles	TRF0105-D TRF0105-M TRF0105-R
SureLight® APC anti-6x His	1 mg	AD0059H
U*Light* anti-GST antibody	1 nmole 10 nmoles 100 nmoles	TRF0104-D TRF0104-M TRF0104-R
SureLight® APC anti-GST	1 mg	AD0059G
SureLight® APC anti-c-myc	1 mg	AD0059C
SureLight® APC anti-FLAG	1 mg	AD0059F
U*Light* protein A	1 nmole 10 nmoles	TRF0103-D TRF0103-M
SureLight® APC anti-mouse IgG	1 mg	AD0059M
SureLight® APC anti-rabbit IgG	1 mg	AD0059R
SureLight® APC wheat germ agglutinin (WGA)	1 mg	AD0059W

ORDERING INFORMATION

Labeling reagents

Product	Size	Catalog Number
LANCE Eu-W1024-ITC labeling reagent	100 µg 1 mg	AD0096 AD0013
LANCE Eu-W1284-iodoacetamide labeling reagent	100 µg 1 mg	AD0107 AD0014
LANCE Eu-W8044-DTA labeling reagent	1 mg	AD0020

Microplates

Plate Type	Well Format	Color	Number of Plates	Catalog Number
OptiPlate™	96	White	50 200	6005290 6005299
		Black	50 200	6005270 6005279
	384	White	50 200	6007290 6007299
		Black	50 200	6007270 6007279
	1536	White	50 200	6004290 6004299
½ AreaPlate	96	White	50 200	6005560 6005569
Shallow ProxiPlate™	384	White	50 200	6008280 6008289
		Black	50 200	6008260 6008269

Plate seals

Product	Seals	Catalog Number
TopSeal™-A, clear adhesive seal	100	6005185

Instrumentation

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TR-FRET Technology: Scientific References

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