HUMAN HEALTH

ENVIRONMENTAL HEALTH

UNMATCHED SENSITIVITY
TIME AFTER TIME

DELFIA®
TRF TECHNOLOGY

PerkinElmer®
For the Better

DELFIA® Dissociation-Enhanced Lanthanide Fluorescent Immunoassay

YOUR RESEARCH WILL SHINE WITH THE DELFIA ALTERNATIVE

DELFIA is a proven, robust immunodetection platform based on time-resolved fluorescence (TRF) detection. This detection method, coupled with the unique chemical properties of lanthanide chelates, makes DELFIA a superior alternative to conventional ELISA for research, drug discovery and clinical applications. DELFIA offers a combination of high sensitivity, wide dynamic range and long-lasting stability that traditional ELISA cannot match.

The DELFIA Advantage

High Sensitivity

- Ideal for complex sample matrices; accurately detect femtogram quantities of analyte

Wide Dynamic Range

- Save time and money by eliminating extensive sample preparation and assay repeats

Superior Stability

- Read plates months later after proper storage, with a stable fluorescent signal that is not time-sensitive

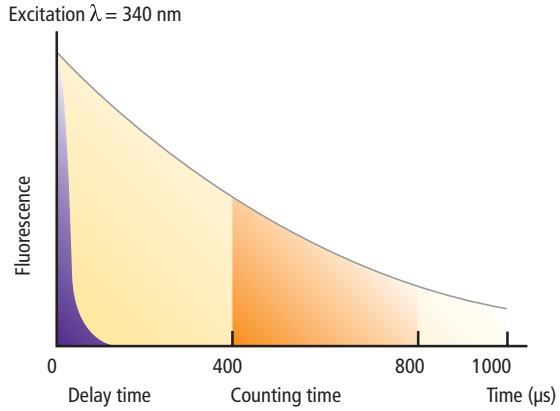
Proven Technology

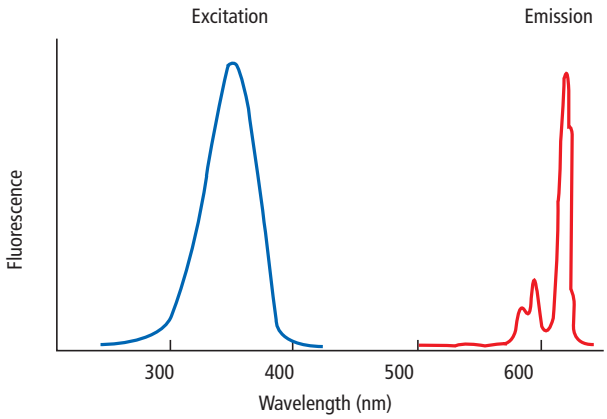
- Backed by thousands of peer-reviewed publications, studying disease diagnostics, neonatal screening, bioweapon detection, and drug discovery (page 8)

Excellent Flexibility

- Miniaturize from 96-well up to a 384-well format
- Multiplex up to three analytes per well
- Widely used in many high performance applications:
  - Immunoassays
  - Cell cytotoxicity
  - Enzyme assays
  - Receptor-ligand binding
  - Adherent cell assays
  - Biodistribution studies
  - Assays for protein-protein, protein-peptide and protein-DNA interactions

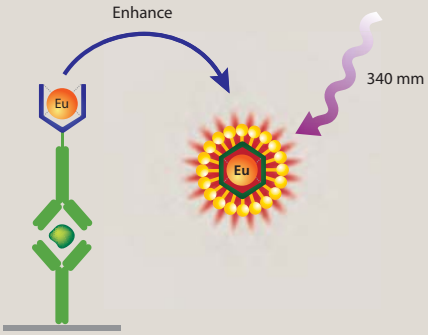
With standard fluorescent detection, reagent and microplate interference can contribute to high background and reduced sensitivity. DELFIA uses the principle of time-resolved fluorometry to remove background interference. Lanthanide chelates possess both long fluorescence decay times and large Stoke's shifts, properties that allow delayed signal measurement at a wavelength with little background interference. In addition, lanthanides emit a stable fluorescent signal that exhibits a sharp emission peak and high fluorescence intensity. Learn more about DELFIA at www.perkinelmer.com/DELFIAadvantage.





Fluorescence from lanthanide chelates may last up to 200,000 times longer than conventional fluorophors (above). Lanthanide chelates exhibit a large Stoke's shift, the difference between the maximum absorption and emission spectra of a fluorophor (below). The sharp emission peak and high fluorescence intensity give DELFIA an advantage over alternative technologies.

The DELFIA Assay Principle



1. The DELFIA assay principle is virtually identical to that of a standard sandwich ELISA; analyte is first captured on a coated microplate, followed by addition of detection antibody to complete the sandwich.

2. Unlike ELISA, DELFIA utilizes a lanthanide chelate-labeled detection antibody, which exhibits minimal fluorescence by itself.

3. An enhancement step unique to DELFIA releases the lanthanide from the antibody complex, producing a new, highly fluorescent lanthanide chelate contained within a protective micelle.

4. The amplified fluorescent signal is detected using time-resolved fluorometry. This detection method removes non-specific interfering fluorescent background signal and provides DELFIA the superior sensitivity it is known for.

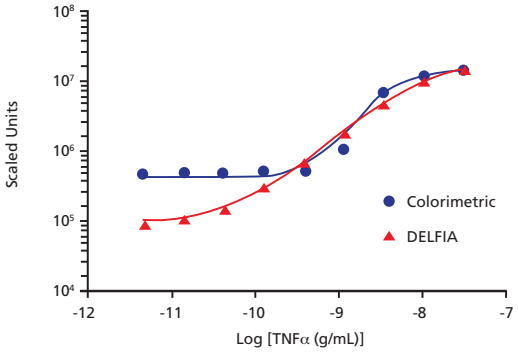
Choose PerkinElmer microplates to optimize your DELFIA results.

GAIN 10X MORE SENSITIVITY OVER ELISA

DELFIA delivers lower detection limits in the femtogram range, sensitivities that are at least 10x greater than standard colorimetric ELISA and comparable to the best chemiluminescent detection systems. DELFIA also offers a broad dynamic range, minimizing the need for time-consuming sample preparation and assay repeats.

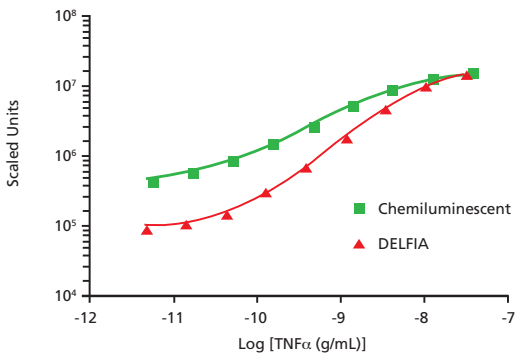
Because DELFIA is not an enzyme-based technology, assay performance is not susceptible to degradation of enzyme conjugate activity or substrate signal. Lanthanide fluorescent signals are strong and stable, providing consistent signal measurement even months after an assay has been completed.

Colorimetric ELISA vs. DELFIA



	DELFIA	ELISA
LDL (pg/mL)	<0.5	371
Dynamic Range	>4 logs	~2 logs

Chemiluminescent ELISA vs. DELFIA



	DELFIA	ELISA
LDL (pg/mL)	<0.5	<0.5
Dynamic Range	>4 logs	>4 logs

DELFIA significantly outperforms colorimetric ELISA (left) and equals the performance of chemiluminescent ELISA (right) in both sensitivity and dynamic range. (Antigen =TNF-α; antibody concentrations were held constant across immunoassay platforms.)

DELFIA Outperforms ELISA at Every Step

	DELFIA	Chemiluminescent ELISA	Colorimetric ELISA
Sensitivity	<0.5 pg/ml	<0.5 pg/ml	<500 pg/ml
Dynamic Range	4-5 logs	4-5 logs	2-3 logs
Experiment Flexibility	Excellent • No stop reaction required • Measurement reads can be taken months later with proper microplate storage	Poor • Luminescent signal declines rapidly; measurements must be made quickly – as soon as 5 minutes after substrate addition	Poor • Stop reaction required • Prompt measurement of colorimetric signal required
Reagent Stability	Excellent • Lanthanide-based assay: Performance characteristics intrinsic to TRF technology	Poor • Enzymatic assay: Performance characteristics dependent on enzyme conjugate quality and activity	Poor • Enzymatic assay: Performance characteristics dependent on enzyme conjugate quality and activity

CONVERT FROM ELISA IN ONE EASY STEP

Converting your ELISA assay to a high-performance DELFIA assay could not be easier, as the assay designs for both are virtually identical. For the standard sandwich ELISA, simply replace your enzyme-conjugated detection antibody with a lanthanide-labeled DELFIA antibody. DELFIA is not enzyme-based, so there is no need for a reaction stop step, and signal measurement is not time-dependent.

The DELFIA Toolbox

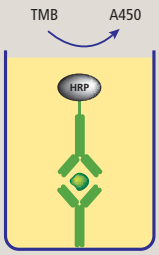
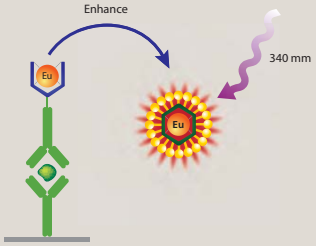
PerkinElmer simplifies your transition to DELFIA with a selection of detection antibodies, labeling reagents, and custom services that make DELFIA conversion a snap. We offer pre-labeled antibodies against several forms of IgG and common protein tags such as GST and HA, as well as pre-coated microplates in an assortment of formats.

Label Your Own Target

If you don't find what you need among our pre-labeled products, DELFIA labeling kits provide all of the necessary reagents to easily label your own molecule of interest. Labeling reactions are simple and robust, and can be used to tag a variety of molecules, including proteins, peptides, oligonucleotides and other small molecules.

DELFIA Eu-labeling Kit

Comparing Assay Processes: ELISA Against PerkinElmer's DELFIA

ELISA	DELFIA
	
Coat plate with capture antibody	
Add sample	
Incubate; wash	
Add HRP-labeled detection antibody	**Add Eu-labeled detection antibody**
Incubate; wash	
Add substrate; monitor closely	**Add Enhancement Solution**
Stop reaction	**(No stop reaction required)**
Read absorbance promptly	**Measure TRF**

CONVENIENT, NON-RADIOMETRIC CYTOTOXICITY & PROLIFERATION DETECTION

Long considered the gold standard for cytotoxicity, proliferation, and ligand binding studies, radiometric assays offer excellent sensitivity and reliability but with increased safety risks, disposal costs, and limited reagent shelf-life. DELFIA provides the same consistent high performance but without the disadvantages of radiochemical use. We offer DELFIA cytotoxicity and proliferation kits as well as a selection of Europium-labeled ligands, which can be used to convert your traditional radiometric assays to DELFIA.

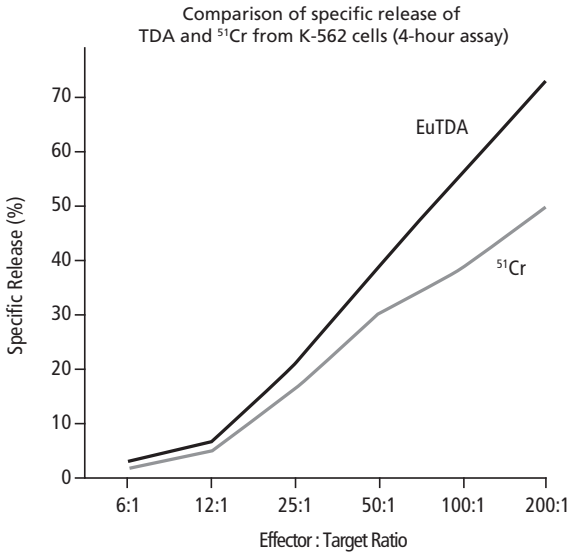
The DELFIA Cytotoxicity Kit: Remove the Radioactivity, Not the Performance

The DELFIA Cytotoxicity assay closely mimics the traditional ^{51}Cr-release protocol while providing sensitivity equivalent to the chromium-based assay, making it an ideal non-radiometric alternative.

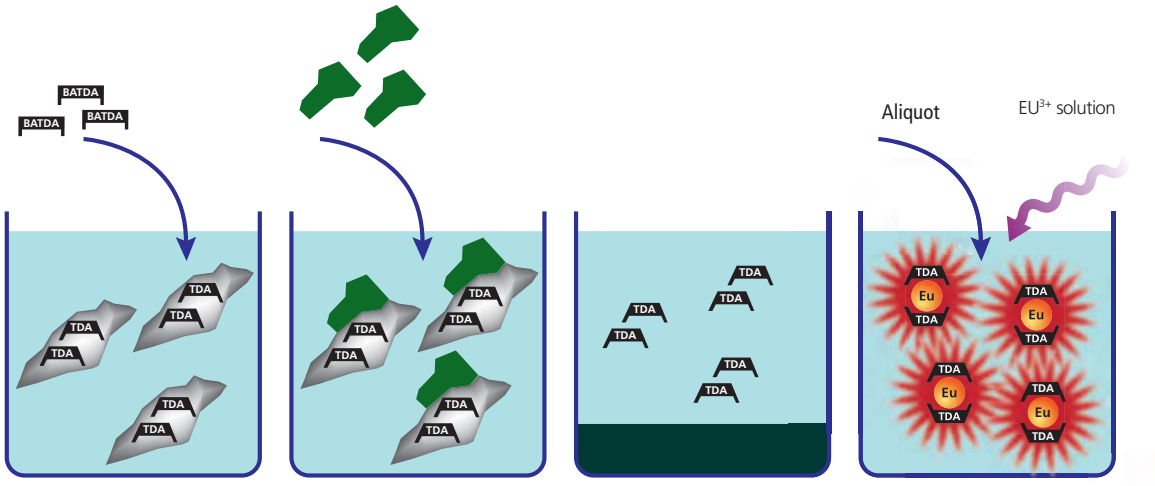
DELFIA Cytotoxicity Kit

KEY BENEFITS

- Sensitive – strong specific release; detect as few as 40 cells/well
- Time-saving – rapid cell loading; quick release of label; reduce assay time by as much as 50%
- Convenient – stable fluorescent signal; longer product shelf-life than ^{51}Cr
- Non-Radioactive



The DELFIA Cytotoxicity assay exhibits strong specific release and its sensitivity correlates well with the ^{51}Cr-release assay.



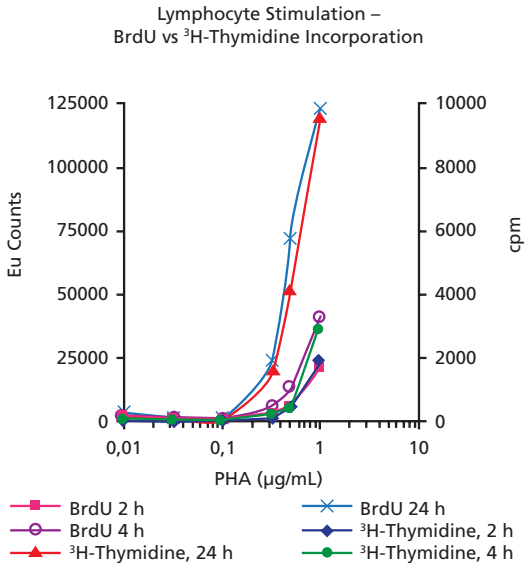
The DELFIA Cytotoxicity assay takes advantage of a fluorescence enhancing ligand (BATDA) which crosses the cell membrane passively, enabling target cell loading that is both rapid and gentle. Once inside the cell, the ligand is immediately hydrolyzed by cellular esterases to generate a hydrophilic molecule (TDA) that can no longer penetrate the membrane. Cytolysis releases TDA into the supernatant to form a highly fluorescent lanthanide chelate with Europium; cytolysis levels are proportional to the amount of fluorescent signal produced.

The DELFIA Cell Proliferation Kit: Highly Sensitive Detection of DNA Synthesis

Historically, the measurement of tritiated thymidine incorporation into newly synthesized DNA has been the method of choice for cell proliferation testing. The DELFIA Cell Proliferation assay couples a similar concept, 5-bromo-2-deoxyuridine (BrdU) incorporation, with highly sensitive Europium-based TRF detection to produce an assay of equivalent performance, all in a non-radioactive format.

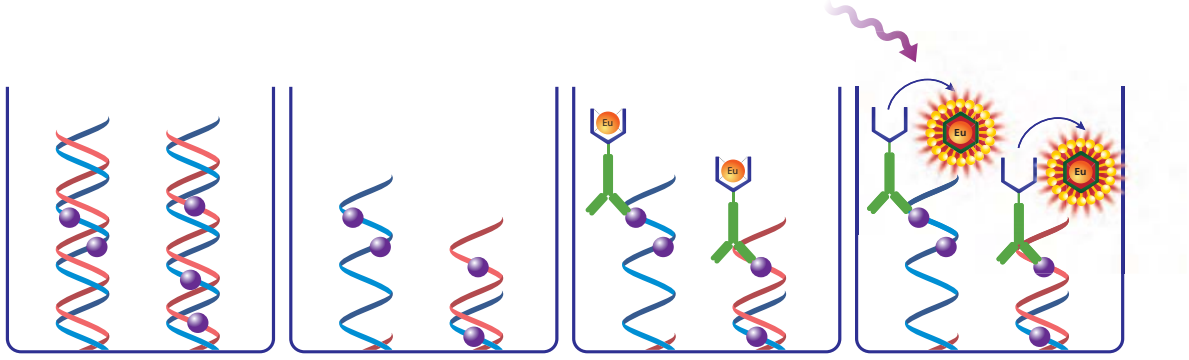
KEY BENEFITS

- Sensitive – detect <100 cells after 2 hour BrdU incubation; 5-fold more sensitive than MTT colorimetric assay
- Time-saving – shorter incubation times
- Flexible – can be used for adherent or suspension cells, as well as for long-term incorporation studies
- Convenient – stable fluorescent signal; longer product shelf-life than tritiated thymidine
- Non-Radioactive



The DELFIA Cell Proliferation assay strongly correlates with the ^{3}H incorporation assay under all experimental conditions. Shown is the proliferative effect of PHA on human lymphocytes.

DELFIA Cell Proliferation Kit



Cells are incubated with the non-radioactive pyrimidine analog BrdU to allow its incorporation into newly synthesized DNA in place of thymidine. Subsequently, Europium-labeled anti-BrdU antibodies are used to detect the level of BrdU incorporation via time-resolved fluorescence, a measurement used as an accurate indicator of cell proliferation.

PROVEN VALUE, MULTIPLE APPLICATIONS

DELFIA is an extremely flexible assay platform used for such applications as: immunoassays, cell cytotoxicity, apoptosis, and proliferation studies, enzyme assays, and assays to detect posttranslational modifications and biomolecular interactions. DELFIA can be used for both biochemical and cell-based assays, from low-throughput diagnostics to higher throughput secondary screens (references follow).

Application	Excerpt	Reference
ELISA Conversion	"Compared to enzyme-linked immunosorbent assays and bioassays, the sensitivity and range of measurement were significantly increased by applying the DELFIA systems to TNF alpha and IL-6. TNF alpha was measurable from 100 fg/ml to 10 ng/ml with the TNF alpha-DELFIA and IL-6 was measurable from 100 fg/ml to 1 ng/ml with the IL-6-DELFIA."	Ogata, A. et al., J Immunol Methods. 1992 Apr 8; 148 (1-2):15-22.
ELISA Conversion	"The method allows measurement of low MT levels that are undetectable using current radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) protocols…"	Butcher H. et al., J Immunol Methods. 2003 Jan 15; 272(1-2): 247-256.
ELISA Conversion	"DELFIA TRF assays are significantly better in terms of sensitivity, linear range, and run time than standard capture ELISAs and should facilitate early detection of potential biological warfare agents in clinical and environmental samples."	Peruski, AH. et al., J Immunol Methods. 2002 May 1;263 (1-2):35-41.
ELISA Conversion	"The TR-FIA method was comparable to the ELISA but had higher sensitivity and required only one-tenth as much sample."	Daijo, J.E. et al., J Pharm Biomed Anal. 1999 Mar; 19(3-4):335-42.
ELISA Conversion	"The DELFIA enhanced the sensitivity of a mouse IL-2 assay 8- to 27-fold, and a human GM-CSF assay 10-fold, as compared to colorimetric ELISA. The increase in sensitivity allows for the use of lower sample volumes per well, and the ability to run more assays per supernatant sample."	Allicotti, G. et al., J Immunoassay Immunochem. 2003; 24(4):345-58.
Cytotoxicity	"Target cells are rapidly labeled when incubated with BATDA, TDA is released from target cells faster than ^{51}Cr, the spontaneous release permits a short-term release assay to be set up and the detection of EuTDA is fast (5 min/96 well plate)."	Blomberg, K. et al., J Immunol Methods. 1996 Jun 21;193(2):199-206.
Ligand-Receptor Binding	"Thus, Eu-labeled peptides present an attractive alternative for commonly used radiolabeled ligands in biological studies in general and in receptor assays in particular."	Mazor, O. et al., Anal Biochem. 2002 Feb 1; 301(1):75-81.
Ligand-Receptor Binding	"These lanthanide-based assays provide superior results with higher throughput and eliminate the need for radioactive waste disposal; hence, they are appropriate for high-throughput screening of ligand libraries."	Handl, H.L. et al., Anal Biochem. 2005 Aug 15;343(2):299-307.
Kinase assays	"This assay provides a highly sensitive, nonradioactive readout of receptor phosphorylation."	Waddleton, D. et al., Anal Biochem. 2002 Oct 1;309(1):150-157.
Biodistribution	"This method offers distinct advantages over traditional techniques employing radioisotopes since it has greater sensitivity, no half-life limitations and no radioactive or hazardous waste disposal."	Neville, M.E. et al., Cytokine. 2000 Nov;12(11):1702-1711.

CHOOSE YOUR ONE STOP DELFIA SOLUTION

Choose from a wide offering of high performance microplates and detection instruments designed to meet all of your DELFIA application needs. When measuring DELFIA Eu-fluorescence on a 96-well plate, these instruments allow a detection limit of better than 10 amol Eu/well with a measurement time of about 2 minutes/plate. With 384-well plates, a detection limit better than 5 amol Eu/well is achieved with a reading time of 3 and 5 minutes on the EnVision® Multilabel Plate Readers and VICTOR™ *X* Multilabel Plate Readers, respectively.

For ultra-fast processing of 384-well plates, the ultraHTS ViewLux™ microplate imager allows detection of all samples on a microplate simultaneously with a detection limit of 50 amol/well.

EnVision Multilabel Plate Reader

VICTOR *X* Multilabel Plate Readers

ultraHTS ViewLux Microplate Imager

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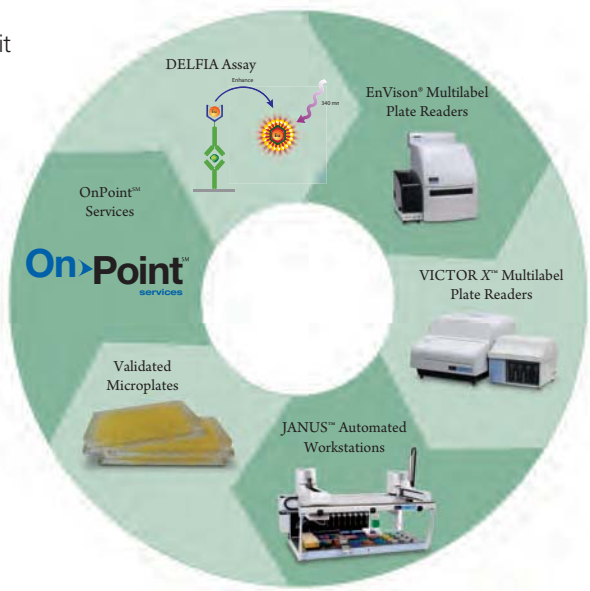

ORDERING INFORMATION

ELISA Conversion

To create your own high-performance DELFIA assay, simply choose a component from each of the following: a detection antibody directed against your primary antibody of interest; a capture microplate; assay buffer for sample dilution, BSA for blocking, and wash buffer; and enhancement solution for signal development. All components have been specifically designed to provide optimum performance when used together in a DELFIA assay.

DELFIA Detection Antibodies/Streptavidin		
Product Description	**Size**	**Part Number**
Eu-N1 labeled streptavidin	250 µg	1244-360
Tb-N1 labeled streptavidin	50 µg/1 mg	AD0047/AD0048
Sm-N1 labeled streptavidin	50 µg/1 mg	AD0049/AD0050
Eu-N1 labeled goat anti-rabbit IgG	200 µg/1 mg	AD0105/AD0106
Eu-N1 labeled rabbit anti-mouse IgG	50 µg/1 mg	AD0124/AD0207
Eu-N1 labeled anti-human IgG	100 µg	1244-330
Eu-N1 labeled anti-HA	50 µg/1 mg	AD0054/AD0053
Eu-N1 labeled anti-6xHis	50 µg/1 mg	AD0108/AD0109
Eu-N1 labeled anti-c-myc	50 µg/1 mg	AD0112/AD0113
Eu-N1 labeled anti-GST	50 µg/1 mg	AD0250/AD0251

DELFIA Microplates		
Clear plates, 8 x 12 strip-wells	60 plates	1244-550
Yellow Plates, 96-well	60 plates	AAAND-0001
Streptavidin-coated clear plates, 96-well	10 plates	4009-0010
Streptavidin-coated yellow plates, 96-well	10 plates	AAAND-0005
Streptavidin-coated white plates, 384-well	10 plates	CC11-H10
Anti-mouse-coated clear plates, 8 x 12 strip-well	10 plates	4007-0010
Anti-rabbit-coated yellow plates, 96-well	10 plates	AAAND-0004
Anti-rabbit-coated clear plates, 8 x 12 strip-well	10 plates	4008-0010

DELFIA Buffers		
Assay Buffer	50/250/1000 mL	1244-106/1244-111/4002-0010
5X Assay Buffer, detergent-free	250 mL	CR85-100
Hybridization Buffer	50 mL	4006-0010
25X Wash Concentrate	250/1000 mL	1244-114/4010-0010
BSA (7.5%, DTPA-purified)	50 mL	CR84-100

DELFIA Detection Reagents and Standards		
Enhancement Solution	50/250/1000 mL	1244-104/1244-105/4001-0010
Enhancer	50 mL	C500-100
Inducer	250 mL	4013-0010
Europium Standard Solution	50 mL	B119-100
Samarium Standard Solution	50 mL	B115-100
Terbium Standard Solution	50 mL	C558-100

For more detailed technical information regarding DELFIA technology, protocols, and applications, please visit PerkinElmer's Reagents Knowledge Base at: www.perkinelmer.com/tsreagents

DELFIA Labeling Reagents		
Product Description	**Size**	**Part Number**
Eu-Labeling Kit (Eu-N1 ITC chelate)	0.2 mg	1244-302
Eu-N1-ITC chelate & Eu standard	1 mg/20 mg	1244-301/AD0001
Eu-N1-iodoacetamido chelate & Eu standard	1 mg	AD0002
Eu-N1-amino chelate & Eu standard	1 mg	AD0003
Eu-N1-DTA chelate & Eu standard	1 mg	AD0004
Sm-Labeling Kit (Sm-N1 ITC chelate)	0.2 mg	1244-303
Sm-N1-ITC chelate & Sm standard	1 mg	AD0005
Sm-N1-iodoacetamido chelate & Sm standard	1 mg	AD0006
Sm-N1-DTA chelate & Sm standard	1 mg	AD0008
Tb-N1-ITC chelate & Tb standard	1 mg	AD0009
Tb-N1-iodoacetamido chelate & Tb standard	1 mg	AD0010
Tb-N1-DTA chelate & Tb standard	1 mg	AD0012
Eu-DTPA-ITC chelate & Eu standard	1 mg	AD0021
Eu-DTPA-iodoacetamido chelate & Eu standard	1 mg	AD0022
Eu-DTPA-amino chelate & Eu standard	1 mg	AD0023
Eu-DTPA-DTA chelate & Eu standard	1 mg	AD0024
Sm-DTPA-ITC chelate & Sm standard	1 mg	AD0025
Tb-DTPA-ITC chelate & Tb standard	1 mg	AD0029

DELFIA Cell Cytotoxicity and Cell Proliferation Reagents		
Cytotoxicity Kit	960 assays	AD0116
Eu solution for AD0116	200 mL	4010-0010
BATDA labeling reagent for AD0116	50 µL	4001-0010
Lysis Buffer for AD0116	30 mL	C500-100
Cell Proliferation Kit	960 assays	AD0200

DELFIA Ligand-Receptor Binding Assay Reagents		
Eu-labeled interleukin-8	160 pmol/700 pmol	AD0213/AD0214
Eu-labeled galanin	200 pmol/800 pmol	AD0215/AD0216
Eu-labeled EGF	350 pmol/1400 pmol	AD0217/AD0218
Eu-labeled neurotensin	250 pmol/750 pmol	AD0219/AD0220
Eu-labeled Substance P	200 pmol/800 pmol	AD0223/AD0224
Eu-labeled NDP-aMSH	200 pmol/800 pmol	AD0225/AD0226
Eu-labeled TNFa	600 pmol	CR400-600
L*R binding buffer concentrate	250 mL	CR134-250
L*R wash solution concentrate	250 mL	CR135-250

DELFIA Kinase Assay Reagents		
Tyrosine Kinase kit	2 X 96 wells	AD0122
Eu-labeled anti-phosphotyrosine antibody (P-Tyr-100)	50 µg/1 mg	AD0159/AD0160
Eu-labeled anti-phosphotyrosine antibody (PY20)	50 µg/1 mg	AD0038/AD0039
Eu-labeled anti-phosphotyrosine antibody (PT66)	50 µg/1 mg	AD0040/AD0041
Eu-labeled antiphosphothreonine antibody	10 µg	AD0092
Eu-labeled anti-phosphoserine antibody	10 µg	AD0185
Eu-labeled anti-phospho-(Ser) 14-3-3 motif antibody	10 µg	AD0189

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

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