LANCE™ Eu-labeled antibodies for serine/threonine kinase assays

For Research Use Only

These instructions for use apply to the following reagents:

AD0094	LANCE Eu-W1024 phospho-threonine antibody	10 µg vial
AD0095	LANCE Eu-W1024 phospho-threonine antibody	1 mg vial
AD0099	LANCE Eu-W1024 phospho-threonine-proline antibody	10 µg vial
AD0100	LANCE Eu-W1024 phospho-threonine-proline antibody	1 mg vial
AD0176	LANCE Eu-W1024 phospho-serine/threonine antibody	10 µg vial
AD0178	LANCE Eu-W1024 phospho-serine/threonine-phenylalanine antibody	10 µg vial
AD0180	LANCE Eu-W1024 phospho-serine/threonine-proline antibody	10 µg vial
AD0182	LANCE Eu-W1024 phospho-PKA substrate antibody	10 µg vial
AD0184	LANCE Eu-W1024 phospho-Akt substrate antibody	10 µg vial
AD0186	LANCE Eu-W1024 phospho-serine antibody	10 µg vial
AD0188	LANCE Eu-W1024 phospho-(Ser) PKC substrate antibody	10 µg vial
AD0190	LANCE Eu-W1024 phospho-(Ser) 14-3-3 binding motif antibody	10 µg vial
AD0192	LANCE Eu-W1024 phospho-(Ser) 14-3-3 binding motif 4E2 antibody	10 µg vial

INTRODUCTION

Europium (Eu)-labeled phospho-serine/threonine antibodies are suitable for setting up non-radioactive serine/threonine kinase assays. The antibodies can be used for detection of phosphorylation with LANCE™ assay technology.

LANCE phospho-Ser/Thr antibodies are intended for setting up homogeneous time-resolved fluorescence resonance energy transfer (TR-FRET) based assays using Eu-chelate label as a donor and APC-labeled reagent as an acceptor. As a simple and rapid assay, LANCE is well suited for automation and miniaturization in High Throughput Screening (HTS).

VIAL CONTENT

LANCE Eu-labeled phospho-Ser/Thr antibodies are supplied as ready-for-use solutions in 50 mmol/L Tris-HCl buffered saline with < 0.1% sodium azide as preservative and 0.1% bovine serum albumin (BSA). One vial contains either 10 µg or 1 mg Eu-labeled antibody; see vial label for the quantity.

Eu-labeled phospho-threonine and phospho-threonine-proline antibodies are supplied in 50 mmol/L Tris-HCl buffered saline containing 5% glycerol (prod. nos. AD0094, AD0095, AD0099 and AD0100).

STORAGE

Store the reagents as such at -20 - +8°C depending on the protein. See label on the vial for the recommended storage temperature. Before use dilute the reagents with a buffer having neutral pH to obtain an appropriate concentration for each assay. Avoid using phosphate buffer or buffers containing high concentrations of chelating agents. Do not store diluted reagents.

NOTE: For maximum recovery of the product, centrifuge or shake down the original vial prior to removing the cap.

Avoid repeated freezing and thawing of the product during storage.

WARNINGS AND PRECAUTIONS

LANCE phospho-Ser/Thr antibodies are intended for research use only.

Reagents contain sodium azide (NaN_3) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Disposal of all waste should be in accordance with local regulations.

ANTIBODY SPECIFICITY

The specificity is given by the suppliers of the antibodies, it has not been tested by PerkinElmer Life Sciences, Wallac Oy. Some of the antibodies recognize several different sequences, and therefore it is recommended to screen several antibodies in order to find an appropriate Eu-labeled phospho-Ser/Thr antibody.

Phospho-threonine antibody AD0094 and AD0095 (supplied by Cell Signaling Technology)

Phospho-threonine antibody is an affinity-purified rabbit polyclonal IgG. The antibody binds threonine-phosphorylated sites in a manner largely independent of the surrounding amino acids sequence. It recognizes a wide range of threonine-phosphorylated peptides and proteins.

Phospho-threonine-proline antibody AD0099 and AD0100 (supplied by Cell Signaling Technology)

Phospho-threonine-proline antibody (P-Thr-Pro-101) is an affinity-purified mouse IgM monoclonal antibody. The antibody recognizes phosphorylated threonine only when followed by proline. It reacts with proteins/peptides phosphorylated on Thr-Pro in a highly context-independent fashion (reactivity is largely independent of the surrounding amino acid sequences).

Phospho-serine/threonine antibody AD0176 (supplied by Upstate)

Phospho-serine/threonine antibody is a mixture of two mouse monoclonal IgG antibodies with broad immunoreactivity for proteins containing phosphorylated serine and phosphorylated threonine residues. It exhibits negligible reactivity with unphosphorylated serine and threonine and is non-reactive with phosphorylated tyrosine.

Phospho-serine/threonine-phenylalanine antibody AD0178 (supplied by Cell Signaling Technology)

Phospho-serine/threonine-phenylalanine antibody is an affinity-purified rabbit polyclonal antibody. It detects phosphorylated serine or threonine with tyrosine, tryptophan, or phenylalanine at –1 position or phenylalanine at +1 position.

Phospho-serine/threonine-proline antibody AD0180 (supplied by Upstate)

Phospho-serine/threonine-proline antibody is a mouse monoclonal IgG antibody. It recognizes a phosphorylated epitope found in phosphoproteins such as MAP2, HSP70, cdc25, and DNA topoisomerase IIα, most of which are phosphorylated at the onset of mitosis. The number of phosphoproteins recognized by the antibody varies from species to species and with cell type.

Phospho-PKA substrate antibody AD0182 (supplied by Cell Signaling Technology)

Phospho-PKA substrate antibody is an affinity-purified rabbit polyclonal antibody. It is highly specific for phosphorylated threonine with arginine at the –3 position. It also recognizes phospho-serine with arginine at the –2 and –3 position.

Phospho-Akt substrate antibody AD0184 (supplied by Cell Signaling Technology)

Phospho-Akt substrate antibody is an affinity-purified rabbit polyclonal antibody. It is highly specific for phosphorylated threonine preceded by arginine at positions –5 and –3. Some cross-reactivity is observed for phospho-serine with arginine at positions –5 and –3 or –3 and –2.

Phospho-serine antibody AD0186 (supplied by Zymed)

Phospho-serine antibody is an affinity-purified rabbit polyclonal antibody. It reacts specifically with proteins containing phosphorylated serine residues. Recognition of proteins containing phosphorylated serine by this antibody is independent of neighboring amino acids and species of origin of the phosphorylated protein. This antibody is specific for phosphoserine-containing proteins and shows no significant cross-reactivity to proteins phosphorylated on threonine or tyrosine residues.

Phospho-(Ser) PKC substrate antibody AD0188 (supplied by Cell Signaling Technology)

Phospho-(Ser) PKC substrate antibody is an affinity-purified rabbit polyclonal antibody. It detects phosphorylated serine residues with arginine or lysine at the –2 and +2 position, and a hydrophobic residue at the +1 position.

Phospho-(Ser) 14-3-3 binding motif antibody AD0190 (supplied by Cell Signaling Technology)

Phospho-14-3-3 binding motif antibody is an affinity-purified rabbit polyclonal antibody. It detects phosphorylated 14-3-3 binding proteins which contain phosphorylated serine surrounded by proline at the +2 position and arginine or lysine at the –3 position. It weakly cross-reacts when phospho-threonine replaces phospho-serine in this motif. The antibody also recognizes the motif containing phospho-serine surrounded by phenylalanine at the +1 position and arginine at the –3 position. Binding is phospho-specific and largely independent of the surrounding amino acid sequence.

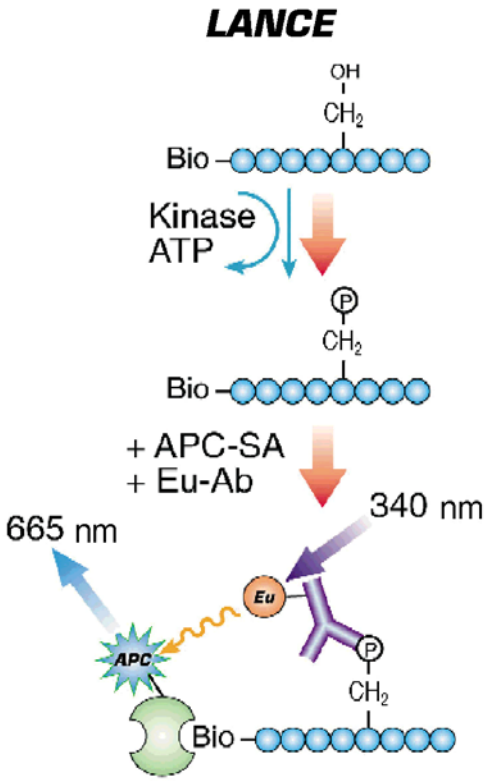
Phospho-(Ser) 14-3-3 binding motif 4E2 antibody AD0192 (supplied by Cell Signaling Technology)

Phospho-14-3-3 binding motif antibody is a mouse monoclonal IgG antibody. It detects phosphorylated 14-3-3 binding proteins which contain phosphorylated serine surrounded by proline at the +2 position and arginine or lysine at the –3 position. It weakly cross-reacts with analogous sequences containing phospho-threonine instead of phospho-serine in this motif, and with sequences containing phospho-serine surrounded by phenylalanine at the +1 position and arginine or lysine at the –3 position. Binding is phospho-specific and largely independent of the surrounding amino acid sequence.

ASSAY PRINCIPLES

LANCE kinase assay is a homogeneous method based on time-resolved detection of energy transfer (TR-FRET) between a highly fluorescent Eu-chelate with a long decay time and allophycocyanin-labeled streptavidin (SA-APC).

For LANCE TR-FRET assays it is recommended that the molar concentration of the APC-labeled component is equal to or exceeds the concentration of the biotinylated reagent to which it is binding. For Ser/Thr kinase assay see the example below. However, the optimal signal-to-noise ratio should be determined by making serial dilutions of the above mentioned reagents as well as the influence of adding bovine serum albumin or suitable detergents for each assay separately.



EXAMPLE ASSAY

This example assays does not include an enzyme assay. Synthetic phosphorylated and unphosphorylated peptide substrates have been used.

LANCE assay was carried out in 50 mmol/L Tris-HCl, pH 7.8 containing 0.5% BSA. The reaction was set up as follows: 100 nmol/L substrate (with 10% and 0% phosphorylation), 1 nmol/L Eu-W1024 labeled antibody and 50 nmol/L SA-APC. The reaction mixtures were incubated in a white 384-well plate, 60 µL/well, for 30 minutes and the plate was measured on a VICTOR multilabel counter using the factory-set LANCE protocol.

Results:

Eu-Ab	Blank signal			Specific signal			S/B
	counts	average	CV%	counts	average	CV%	
1	2805 2901	2853	2.4	3106 2921	3014	4.3	1
2	3914 3670	3972	4.5	19674 18587	19131	4.0	5

LANCE settings for various VICTOR models

A typical LANCE measurement in TR-FRET includes measuring of both donor (Eu at 615 nm) and acceptor (APC at 665 nm) emissions using identical counting parameters except the filters. Both values are needed if quench correction is required (for more detailed information please refer to Application note "Quench Correction for TR-FRET").

Counting parameters for LANCE labels are instrument dependent because each instrument is individually calibrated. The following table is for your reference.

When using europium as a donor and APC as an acceptor the following parameters should be used. First measurement is done with Eu filter (615) and second with 665 filter.

Parameter	VICTOR	VICTOR LANCE Upgraded	VICTOR^2	VICTOR^2 HTS (LANCE model)	VICTOR^{2}V (LANCE protocol 615/665)
Flash Energy area	copy Eu	copy Eu	copy Eu	copy Eu	copy Eu
Flash Energy level	copy Eu	copy Eu	copy Eu	copy Eu	copy Eu
Excitation filter	340	'390'	320	340	340
Integrator cap.	1	1	1	1	1
Integrator level	copy Eu	copy Eu	copy Eu	copy Eu	copy Eu
Emission filter	1) 615 2) 665	1) 615 2) 665	1) 615 2) 665	1) 615 2) 665	1) 615 2) 665
Delay time	70 µs	50 µs	50 µs	50 µs	50 µs
Window	200	100	100	100	100
Cycle	1000	1000	1000	1000	1000

PROCEDURAL NOTES FOR LANCE ASSAYS

1. Two-incubation assay protocols should be created for samples containing citrate, EDTA or any other chelating agent because of their chelating effect on the lanthanide when mixed with the lanthanide-labeled protein. Use of PerkinElmer Labeling Service is recommended for assays where the labeled reagent is exposed to a pH < 7 or to chelating agents such as EDTA or citrate (at a concentration > 0.05 mmol/L).

2. Streptavidin conjugated to allophycocyanin (SA-APC) is used as energy acceptor. The concentration of SA-APC used in each assay has to be optimized separately. As a general rule for LANCE kinase assays, the streptavidin concentration should be 0.5-1 times the concentration of the biotinylated substrate. Different SA-APC products are offered, one product with one streptavidin coupled to APC and another with 5 streptavidins coupled to one APC.

3. The avoidance of europium contamination and resulting high fluorescent background demands high standard pipetting techniques. Avoid contaminating pipettes with Eu-labeled reagents or labeled proteins.

WARRANTY

Purchase of the product gives the purchaser the right to use this material in his own research, development, and investigational work. The product is not to be injected into humans or used for diagnostic procedures. Wallac Oy reserves the right to discontinue or refuse orders to any customer who plans to use these products for any other purposes.

Wallac Oy does not warrant or guarantee that the product is merchantable or satisfactory for any particular purpose, nor free from any claim of foreign or domestic patent infringement by a third party, and there are no warranties, expressed or implied, to such effect. Wallac Oy will not be liable for any incidental, consequential or contingent damages involving their use including damages to the property or personal injuries.

All information supplied with the product and technical assistance given is believed to be accurate, but it remains the responsibility of the investigator to confirm all technical aspects of the application. We appreciate receiving any additions, corrections, or updates to information supplied to the customer.

LITERATURE

Tamminen, J., Ahola, T., Toivonen, A., Andersson, K., Hurskainen, P. and Bunker, C. (2001): Screening for specific antibodies for Ser/Thr kinase assays based on time-resolved fluorescence. Paper presented at the 7th Annual Conference of The Society for Biomolecular Screening. Baltimore, MD, Sept. 2001, Abs #10094.

Eu-labeled anti-phosphoserine/threonine antibody specificity test service. Wallac Model Report 1599-9748.

PATENTS

Both the chemical structure and the LANCE type assays are covered by following patents:

PCT WO 98/15830
US 4,920,195
US 5,830,769

June 2002

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