Generic LANCE™ Reagents

For Research Use Only

These instructions for use apply to the following reagents:

AD0060	LANCE Eu-W8044 streptavidin	50 µg vial
AD0061	LANCE Eu-W8044 streptavidin	1 mg vial
AD0062	LANCE Eu-W1024 streptavidin	50 µg vial
AD0063	LANCE Eu-W1024 streptavidin	1 mg vial
AD0066	LANCE Eu-W1024 PY20 antibody	50 µg vial
AD0067	LANCE Eu-W1024 PY20 antibody	1 mg vial
AD0068	LANCE Eu-W1024 PT66 antibody	50 µg vial
AD0069	LANCE Eu-W1024 PT66 antibody	1 mg vial
AD0070	LANCE Eu-W1024 Protein G	50 µg vial
AD0071	LANCE Eu-W1024 Protein G	1 mg vial
AD0074	LANCE Eu-W1024 anti-human IgG antibody	50 µg vial
AD0075	LANCE Eu-W1024 anti-human IgG antibody	1 mg vial
AD0076	LANCE Eu-W1024 anti-mouse IgG antibody	50 µg vial
AD0077	LANCE Eu-W1024 anti-mouse IgG antibody	1 mg vial
AD0082	LANCE Eu-W1024 anti-rabbit IgG antibody	50 µg vial
AD0083	LANCE Eu-W1024 anti-rabbit IgG antibody	1 mg vial
AD0084	LANCE Eu-W1024 anti-HA antibody	50 µg vial
AD0085	LANCE Eu-W1024 anti-HA antibody	1 mg vial
AD0110	LANCE Eu-W1024 anti-6xHis antibody	50 µg vial
AD0111	LANCE Eu-W1024 anti-6xHis antibody	1 mg vial
AD0114	LANCE Eu-W1024 anti-c-myc antibody	50 µg vial
AD0115	LANCE Eu-W1024 anti-c-myc antibody	1 mg vial
AD0161	LANCE Eu-W1024 P-Tyr-100 antibody	50 µg vial
AD0162	LANCE Eu-W1024 P-Tyr-100 antibody	1 mg vial
AD0203	LANCE Eu-W1024 P-Tyr-100 antibody	10 µg vial
AD0205	LANCE Eu-W1024 anti-6xHis antibody	10 µg vial
AD0206	LANCE Eu-W1024 anti-c-myc antibody	10 µg vial
AD0211	LANCE Eu-W1024 Protein G	10 µg vial
AD0212	LANCE Eu-W1024 anti-human IgG antibody	10 µg vial
AD0252	LANCE Eu-W1024 anti-GST-antibody	10 µg vial
AD0253	LANCE Eu-W1024 anti-GST-antibody	50 µg vial
AD0254	LANCE Eu-W1024 anti-GST-antibody	1 mg vial

INTRODUCTION

Generic LANCE™ reagents 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. Generic reagents facilitate setting up LANCE assays when there is a limited amount of specific reagents available or the assay reagents are relatively unstable. To enable optimization of both the signal to noise ratio and signal stability, some of the generic reagents are labeled with two different chelates, W1024 or W8044.

VIAL CONTENT

Generic reagents are supplied as ready-for-use solution in 50 mmol/L Tris-HCl buffered saline with < 0.1 % sodium azide as preservative. One vial contains either 10 µg, 50 µg or 1 mg Eu-labeled protein; see label on the vial for the quantity.

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

Generic LANCE reagents 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.

PROTEIN SPECIFICITY

Anti-GST antibody

The antibody is purified from goat serum. The antibody binds to GST protein from *Schistosoma japonicum* expressed from the pGEX vector in *E. coli*.

Anti-human IgG antibody

The products are made from *in vitro* produced and affinity purified mouse monoclonal IgG2b antibody specific for the Fc-part of human IgG. The antibody recognizes all IgG subclasses and cross-reacts with rabbit immunoglobulins.

Anti-mouse IgG antibody

The antibody is an affinity purified rabbit polyclonal antibody which reacts with all mouse IgG subclasses. The reaction with IgG1, IgG2a and IgG2b is somewhat stronger than reaction with IgG3. The antibody also reacts with mouse IgA and IgM.

Cross-reaction with human immunoglobulins is less than 0.2 %, with fetal calf serum less than 0.1 % and with rat serum and rat IgG less than 3 %. Cross-reaction with goat, guinea pig, ox and swine immunoglobulins is less than 1.5 % when determined with ELISA.

Anti-phosphotyrosine antibodies

The PY20 antibody is a mouse monoclonal antibody, IgG2b that binds to phosphorylated tyrosine residues.

The PT66 antibody is an affinity purified IgG1 subclass of mouse monoclonal antibody that binds to phosphorylated tyrosine residues.

The P-Tyr-100 antibody (supplied by Cell Signaling Technology) is a mouse monoclonal IgG1 antibody that binds to phosphorylated tyrosine residues.

Anti-rabbit IgG antibodies

The antibody is an affinity purified polyclonal goat antibody and it reacts with all classes of rabbit immunoglobulins. Cross-reaction with human and mouse immunoglobulins is less than 0.7 %, with ox, rat and swine immunoglobulins and fetal calf serum less than 0.1 % and with guinea pig immunolobulins about 20 % when determined with ELISA.

Protein G

This is a recombinant form of Protein G cloned from *Streptococcus* species and produced in *E. coli*. Protein G binds to the Fc region of IgG by a non-immune mechanism, which is similar to that of Protein A, but has a broader binding range and higher binding affinity.

Protein G binds to all subclasses of human IgG and mouse IgG. In addition it binds to rat, goat, sheep, guinea pig, rabbit, cow, pig and horse antibodies. It does not bind to chicken or cat antibodies, and binds weakly to IgG from dog. Protein G does not bind to human IgA, IgM or serum albumin.

Streptavidin

Streptavidin is produced by *Streptomyces avidinii* and isolated from fermentation filtrates.

Anti-HA antibody

The anti-HA antibody is a purified IgG2b subclass of mouse monoclonal antibody. It recognizes the epitope sequence (YPYDVPDYA) derived from the human influenza hemagglutinin (HA) protein.

Anti-6xHis antibody

The anti-6xHis antibody is a purified mouse IgG1 monoclonal antibody. Monoclonal 6xHis antibody was raised against a polypeptide containing a 6x histidine tag. The antibody has been shown to detect polyhistidine tags localized at the amino- or carboxyl-terminus.

Anti-c-myc antibody

The antibody is a purified mouse IgG1 monoclonal antibody. It recognizes the epitope sequence (EQKLISEEDL), which was derived from the human c-myc protein. The monoclonal antibody against the c-myc epitope does not cross react with other cellular proteins.

LANCE ASSAYS

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 reagent to which it is binding. In development of a binding assay, it can be assumed that the optimal concentration of Eu-labeled generic reagent is close to the concentration of the compound it is binding to. For 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.

Examples of LANCE assay procedures

Tyrosine kinase assay

50 µL of 500 nmol/L biotinylated peptide (KVEKIGEGTYGV VYK) is incubated in a 96-well plate for 3 hours at +30°C with 20 µmol/L ATP and 2 mmol/L Mg^{2+} using 10 units of abl protein tyrosine kinase enzyme (New England Biolabs).

200 µL of detection mixture is added to the same microtitration wells. Detection mixture contains 1 nmol/L LANCE Eu-labeled antiphosphotyrosine antibody (PT66 or PY20) and 2.5 µg/mL Streptavidin-APC in Tris-HCl buffered (pH 7.5) salt solution with 0.1 % BSA.

If the enzyme activity requires the presence of heavy metal cations, such Mn^{2+}, addition of EDTA is required both to stop the enzyme reaction and to avoid ion-pair quenching of Eu chelates. It is recommended to add EDTA just enough to chelate Mn^{2+}, to avoid signal decrease. Typically up to 10 – 15 mmol/L EDTA concentration has been used. Ca^{2+} and Mg^{2+} do not cause any quenching.

Energy transfer signal is measured using 1420-018 HTS VICTOR^{2}™. For protocol parameters see section ”LANCE settings for various VICTOR models”.

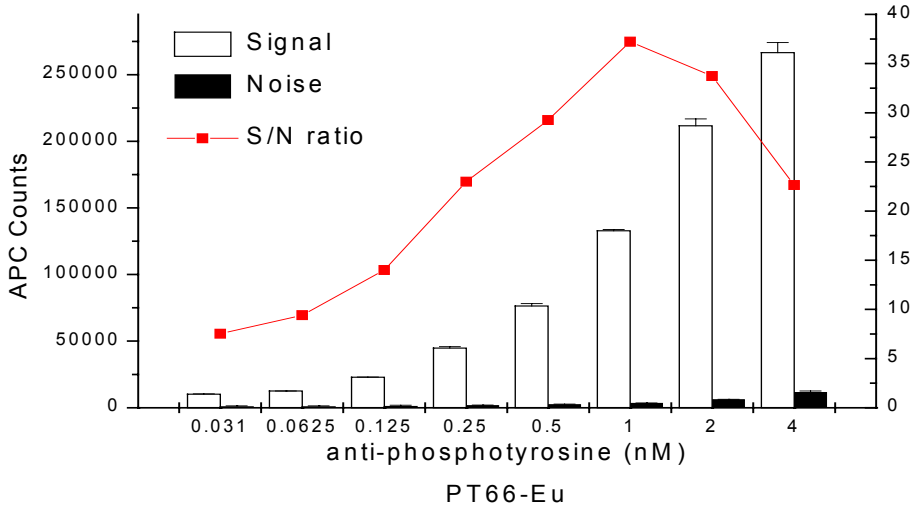

Figure 1. Signal to noise values and Energy Transfer signal with different antibody concentrations using PT66-W1024-Eu. Streptavidin-APC concentration 2.5 µg/mL. Abl kinase enzyme (NEB) 10 units per well.

Signal to noise ratio is typically over 20 but it depends on the kinase enzyme used, reaction time and temperature, substrate and enzyme concentrations. Using 50 nmol/L of phosphorylated peptide (biotinyl-ε-aminocaproyl-Glu-Pro-Gln-Tyr(PO_3H_2)-Glu-Glu-Ile-Pro-Ile-Tyr-Leu-OH) as a control, signal to noise ratio up to 60 has been achieved using LANCE Eu-labeled PT66 antibody as a donor label.

VICTOR is a trademark of PerkinElmer, Inc.

Detection of GST fusion protein using LANCE Eu-labeled anti-GST antibody

200 µL of 1 nmol/L LANCE Eu-labeled anti-GST antibody, 8 µg/mL of streptavidin-APC and 0-5-10-20 nmol/L biotinylated GST fusion protein are mixed in a 96-well plate and incubated at room temperature for 30 - 60 minutes.

Energy transfer signal is measured with LANCE upgraded VICTOR. For protocol parameters see section "LANCE settings for various VICTOR models".

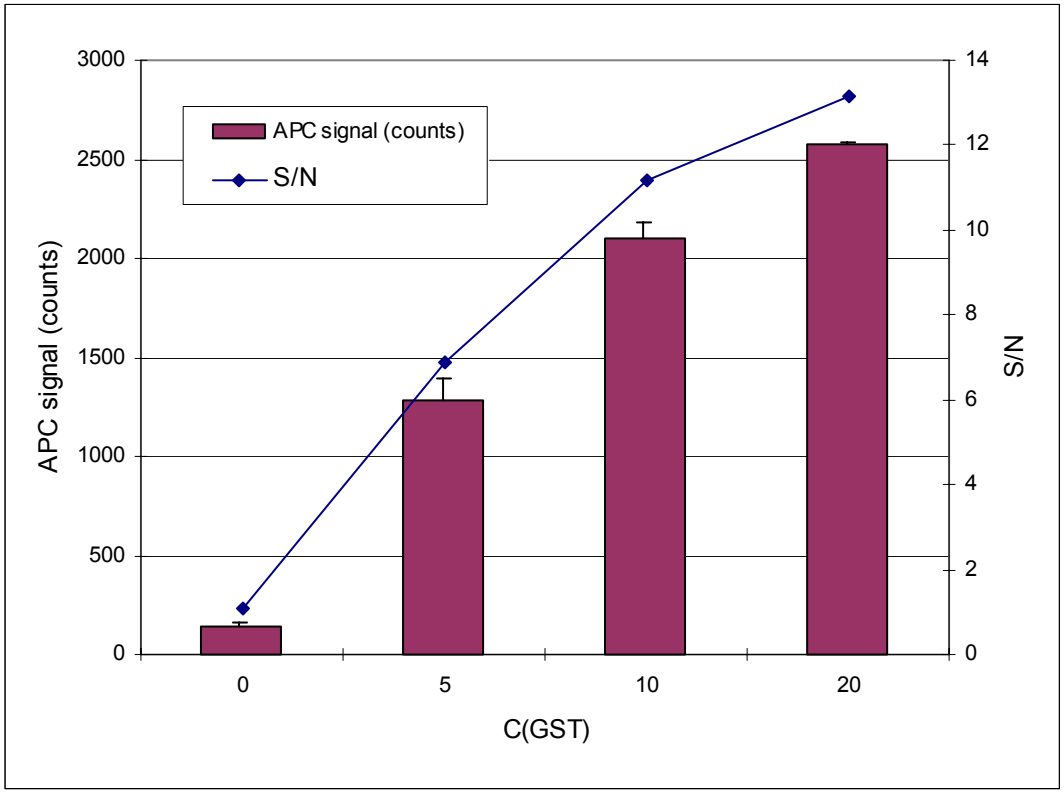

Figure 2. Signal-to-noise values and Energy Transfer signal with different GST protein concentrations using 1 nM GST-W1024-Eu as an energy transfer donor and 24 nM Streptavidin-APC as an acceptor. Incubation was performed at room temperature for 1 h in Nunc black 96-well microplates.

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

For the Generic LANCE Reagents labeled with Eu-W8044 chelate (decay time over 1 ms) extending of cycle time to 4000 µs will improve signal to noise ratio. The extending of cycle time will increase the measurement time.

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. PerkinElmer Life Sciences, Wallac Oy reserves the right to discontinue or refuse orders to any customer who plans to use these products for any other purposes.

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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

Ahola, T., Virtanen, J., Toivonen, A., Hemmilä, I. and Hurskainen, P. (1998): Use of a generic reagent in LANCE TR-FRET assays. Paper presented at the 4th Annual Conference of The Society for Biomolecular Screening. Baltimore, MD, Sept. 1998, Abs SDAT-33.

Boisclaire, M., McClure, C., Hemmilä, I. and Webb, S. (1998): A rapid protein transferase screen based on fluorescence resonance energy transfer. Paper presented at NMHCC International Conference on Assay Development, Screen ´98, San Diego, CA, Jan. 1998.

Moore, K.J., Turconi, S., Miles-Williams, A., Djaballah, H., Hurskainen, P., Hemmilä, I., Harrop, J., Truneh, A., Young, P., Silverman, C., Lyn, S., Murray, K.J. and Pope, A.J. (1998): Development of a homogenous 384-well high throughput screen for novel TNF superfamily receptor – Ligand interactions using time resolved fluorescence. Paper presented at the 4th Annual Conference of The Society for Biomolecular Screening. Baltimore, MD, Sept. 1998, Abs AM-13.

Ollikka, P., Hemmilä, I., Kivelä, P. and Blomberg, K. (1998): Miniaturization of a LANCE assay. Paper presented at the 4th Annual Conference of The Society for Biomolecular Screening. Baltimore, MD, Sept. 1998, Abs AM-14.

PATENTS

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

US 4,925,804
US 5,637,509
PCT WO 87/07955
PCT WO 98/15830
US 4,761,481
US 4,920,195
US 5,032,677
US 5,202,423
US 5,324,825
US 5,457,186
US 5,571,897

Last revision September 2003

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