Generic DELFIA® Reagents

For Research Use Only

These instructions for use apply to the following reagents:

AD0038	DELFIA Eu-N1 PY20 antibody	50 µg vial
AD0039	DELFIA Eu-N1 PY20 antibody	1 mg vial
AD0040	DELFIA Eu-N1 PT66 antibody	50 µg vial
AD0041	DELFIA Eu-N1 PT66 antibody	1 mg vial
AD0046	DELFIA Eu-N1 biotinylated peptide	50 µg vial
AD0047	DELFIA Tb-N1 streptavidin	50 µg vial
AD0048	DELFIA Tb-N1 streptavidin	1 mg vial
AD0049	DELFIA Sm-N1 streptavidin	50 µg vial
AD0050	DELFIA Sm-N1 streptavidin	1 mg vial
AD0053	DELFIA Eu-N1 anti-HA antibody	1 mg vial
AD0054	DELFIA Eu-N1 anti-HA antibody	50 µg vial
AD0105	DELFIA Eu-N1 anti-rabbit antibody	200 µg vial
AD0106	DELFIA Eu-N1 anti-rabbit antibody	1 mg vial
AD0108	DELFIA Eu-N1 anti-6xHis antibody	50 µg vial
AD0109	DELFIA Eu-N1 anti-6xHis antibody	1 mg vial
AD0112	DELFIA Eu-N1 anti-c-myc antibody	50 µg vial
AD0113	DELFIA Eu-N1 anti-c-myc antibody	1 mg vial
AD0124	DELFIA Eu-N1 anti-mouse antibody	50 µg vial
AD0159	DELFIA Eu-N1 P-Tyr-100 antibody	50 µg vial
AD0160	DELFIA Eu-N1 P-Tyr-100 antibody	1 mg vial
AD0207	DELFIA Eu-N1 anti-mouse antibody	1 mg vial
AD0250	DELFIA Eu-N1 anti-GST antibody	50 µg vial
AD0251	DELFIA Eu-N1 anti-GST antibody	1 mg vial

INTRODUCTION

Generic DELFIA® reagents are intended for use in dissociation-enhanced time-resolved fluorometric assays. All reagents have been labeled using the DELFIA N1-chelate (Patent 1). They are suitable for highly sensitive end point measurements in separation based assays such as those for detection of protein-protein binding and cell adhesion as well as for immunoassays.

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, 200 µg or 1 mg Eu/Tb/Sm-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. If the background level of the assay tends to increase during the storage, the labeled antibody should be filtered through a 0.2 µm membrane.

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 DELFIA 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-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.

Biotinylated peptide

The biotinylated peptide is 11 aminoacids long.

Streptavidin

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

Anti-rabbit IgG antibody

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.

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.

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.

USE OF LABELED PROTEINS

Lanthanide labeled reagents can be applied in different types of immunoassays, enzyme assays as well as in receptor assays based on solid-phase separation. The design of the assay depends on the analyte, the antibodies, the possibility of using a sandwich type assay or the need to employ a competitive assay-design, the required sensitivity and dynamic range etc.

As a general rule, about 25–100 ng of labeled antibodies per well is enough for non-competitive sandwich-type assays, but the actual optimal level depends on the purity and affinity of the antibodies and the desired signal levels. For competitive assays no general rules can be given and the assay always has to be separately optimized.

The labeled protein as such is practically non-fluorescent. After binding assay DELFIA Enhancement Solution (prod. no. 1244-105) dissociates Eu/Sm/Tb ions from labeled protein into solution, where Eu and Sm ions form highly fluorescent chelates with components of the Enhancement Solution (Patent 2). The strips should be shaken **slowly** for 5 minutes before measuring with the time-resolved fluorometer (the 1420 VICTOR™ or the 1234 DELFIA Research Fluorometer).

For detection of Tb-labeled protein first add 200 µL of Enhancement Solution per well and shake for 5 minutes. In a multilabel assay, first measure Eu and Sm fluorescence, then add 50 µL of the DELFIA Enhancer (prod. no. C500-100) to each well, shake for 5 minutes and measure Tb.

The DELFIA Assay Buffer (prod. no. 1244-106) is optimal for most assays. In some assays, additional components might be needed to overcome cross-reactivity problems. The DELFIA Wash Concentrate (prod. no. 1244-114) is the optimized wash solution to be used specially in immunoassays.

PROCEDURAL NOTES FOR DELFIA 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. When washing the microtitration plate wells, ensure that each well is filled up completely to the top edge. After washing the strips, check that the wells are dry.

   For detailed information on the cleaning and maintenance of the washing device, please refer to the DELFIA Platewash manual.

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

4. The DELFIA Enhancement Solution should be dispensed using the dedicated dispensing unit DELFIA Plate Dispense or Eppendorf Multipette after the Combitip has first been flushed with Enhancement Solution. The same Combitip must not be used for pipetting any other reagent.

   When using the DELFIA Plate Dispense, please refer to the manual.

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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.

PATENTS

The reagents are covered by the following patents on both the chemical structure and the dissociation enhancement principle:

Patent 1. Mikola, H., Mukkala, V-M. and Hemmilä, I. (1987): Eur. Patent No. 139,675.
Mikola, H., Mukkala, V-M. and Hemmilä, I. (1989): US Patent No. 4,808,541.

Patent 2. Hemmilä, I. and Dakubu S. (1982): Eur. Patent No. 64,484.
Hemmilä, I. and Dakubu S. (1982): US Patent No. 4,565,790.

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precisely.