TECHNICAL DATA SHEET

DELFIA®

Caution: For Laboratory Use. A product for research purposes only.

Eu-DTPA Amino Chelate & Europium Standard

Development Grade

Product Number: AD0023

INTRODUCTION:

DELFIA® Europium chelate of 1-(p-aminobenzyl)diethylenetriamine-N^1,N^1,N^2,N^3,N^3-pentaacetic acid (DTPA) is optimized for labelling of small compounds containing at least one carboxyl group. The labelled compound can be used in dissociation-enhanced time-resolved fluorometric assays.

PACKAGE CONTENTS:

1 vial (2 mg, 3 µmol) of Eu-DTPA Amino Chelate
1 vial (0.5 mL) of 100 nmol/L Europium Standard

STORAGE:

The manufacturing date of the chelate is stated on the vial label. Store the chelate at -20°C. Store the standard at +2 - +8°C.

REAGENT RECONSTITUTION:

Dissolve the chelate in distilled water (e.g. in 50 µL giving 60 mmol/L solution).

RECONSTITUTED STABILITY:

For a longterm storage the chelate dissolved in water should be kept at -20°C.

WARNINGS AND PRECAUTIONS:

This labelling reagent is intended for research use only.

The handling of concentrated Eu^{3+}-solutions constitutes a contamination risk, which may cause elevated backgrounds in an assay based on time-resolved fluorometry. Keep the labelling reagents and required accessories separated from the place and accessories where the actual assay is performed.

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

LABELLING OF SMALL COMPOUNDS:

It is sometimes beneficial to introduce a suitable reactive group or a spacer arm to the hapten molecule before the coupling reaction to maintain the biological activity of the molecule. The coupling site, the chemical structure of the linkage and the length of spacer arm between the hapten and the chelate all play an important role in the binding recognition in an assay. An additional factor to be taken into account is the hydrophilic nature of the chelate.

1. Parameters of labelling reaction
   Parameters of labelling reaction include hapten concentration, pH, temperature, reaction time, molar excess of chelate over the molecule.

2. Labelling
   The optimal pH range for the carbodiimide reaction is pH 4.7 – 6.

LABELLING PROCEDURE:

1. Labelling
   The compound to be labelled should be dissolved in 0.5 mol/L MES (or HEPES), pH 5.5 at a concentration of 0.01 – 0.10 mol/L. If necessary, it can first be dissolved in 1,4-dioxane or DMF, and then the buffer solution is added. The amount of the organic solvent should be less than 50 % of the total volume.

   Eu-chelate in water is added at 1.2-fold molar excess compared to carboxylate group.

   Then EDAC is added to the above mentioned solution at a molar excess of 1.2 compared to the carboxylate group. The pH of the reaction mixture should be adjusted to 5.5. The reagents are incubated for 1 - 2 hours.

2. Purification
   Depending on the amount of the starting material, the mixture should first be purified with preparative thin layer chromatography (silica plate) using e.g. acetonitrile:water (4:1) as an eluent and then with HPLC. If only a small amount has been labelled then direct purification with HPLC is possible.

   It is advisable to remove acetonitrile from the purified Eu-labelled molecule to increase its stability.

Superdex[1] 75 HR 10/30 or Superdex Peptide HR 10/30: 0 - 20 % acetonitrile in 0.05 mmol/L NaCl and 0.05 mmol/L TRIS-HCl, pH 8

[1] Superdex is a trademark of Amersham Pharmacia Biotech.

RP C_4 – C_{18} columns: Eluent A: 5 - 10 % acetonitrile in 0.1 mol/L TEAAc, pH 7.5 Eluent B: 40 - 50 % acetonitrile in 0.1 mol/L TEAAc, pH 7.5 Gradient 0 - 60 % B in 30 min.

Table 1. Some suitable columns and eluents for HPLC purification.

There should be dedicated columns for each lanthanide (europium, terbium, samarium, dysprosium) used for labelling. After purification, columns should be decontaminated by washing with 10 mmol/L phthalate buffer (pH 4) containing 0.01 % DTPA. Additionally, before each equilibration and purification step it is preferable to further wash and saturate Superdex columns with BSA of high purity.

CHARACTERIZATION OF LABELLED COMPOUNDS:

To determine the europium content of the labelled compound, dilute it in DELFIA Enhancement Solution (prod. no. 1244-105) and incubate at room temperature (+20 - +25°C) for 30 minutes. The fluorescence is then measured in a time-resolved fluorometer against 100 nmol/L Eu standard (supplied with the chelate) diluted 1:100 in DELFIA Enhancement Solution (1 nmol/L Eu in Enhancement Solution in a clear 96-well plate, 200 µL per well, gives about 1 000 000 cps when measured in 1234 DELFIA Research Fluorometer or 1420 VICTOR™ Multilabel Counter).

FILTRATION:

To remove particles and possible aggregates the labelled compound should be filtered through a 0.22 µm low protein binding membrane.

STORAGE OF LABELLED COMPOUNDS:

To ensure stability, the lanthanide-labelled compounds should be stored at a high concentration and in the absence of chelators or competing metals in the buffer. Temperature during storage is determined by the stability of the hapten. For a long term storage the compound should be in polypropylene tubes at -20°C to -70°C. It is recommended that the labelled compound is stored as a concentrated solution (10 µmol/L or higher).

WARRANTY:

Purchase of this reagent gives the purchaser the right to use this material in his own research. Further distribution of this reagent is expressly prohibited. Purchase of this product implies agreement with these conditions of sale.

LITERATURE:

VICTOR is a trademark of Perkinelmer.

Mikola, H. and Miettinen, P. (1991): Preparation of europium labeled derivatives of cortisol for time-resolved fluoroimmunoassays. Steroids, **56**, 17-21.

Mikola, H., Sundell, A-C. and Hänninen, Elina (1993): Labeling of estradiol and testosterone alkyloxime derivatives with a europium chelate for time-resolved fluoroimmunoassays. Steroids, **58**, 330-334.

PATENT:

The use of reagents is covered by the following patent on the dissociation enhancement principle:

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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