INTRODUCTION

DELFIA® Europium chelate of N\textsuperscript{1}-(p-aminobenzyl)diethylenetriamine-N\textsuperscript{1},N\textsuperscript{2},N\textsuperscript{3},N\textsuperscript{3}-tetraacetic acid is optimized for labeling of small compounds containing at least one carboxyl group. The labeled compound can be used in dissociation-enhanced time-resolved fluorometric assays.

PACKAGE CONTENTS

1 vial (1 mg, 1.6 µmol) of Eu-N1 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 30 µL of distilled water giving 53 mmol/L solution of the chelate) for immediate use. Keep at 0°C (ice bath).

WARNINGS AND PRECAUTIONS

This labeling reagent is intended for research use only.

The handling of concentrated Eu\textsuperscript{3+}-solutions constitutes a contamination risk, which may cause elevated backgrounds in an assay based on time-resolved fluorometry. Keep the labeling 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.

LABELING 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.

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1. Parameters of labeling reaction

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

2. Labeling

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

LABELING PROCEDURE

1. Labeling

The compound to be labeled 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 labeled then direct purification with HPLC is possible.

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

| Superdex¹ 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 |

| RP C₄ – C₁₈ 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 labeling. After purification, columns should be decontaminated by washing with 10 mmol/L phthalate buffer (pH 4) containing 0.01 %

¹ Superdex is a trademark of Amersham Pharmacia Biotech.
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 LABELED COMPOUNDS

To determine the europium content of the labeled compound, dilute it in DELFIA Enhancement Solution (prod. no. 1244-105). 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 labeled compound should be filtered through a 0.22 µm low protein binding membrane.

STORAGE OF LABELED COMPOUNDS

To ensure stability, the lanthanide-labeled 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 labeled 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


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VICTOR is a trademark of PerkinElmer, Inc.
PATENTS

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


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