DELFIA® Tb-N1 DTA Chelate & Terbium Standard

AD0012

For Research Use Only

INTRODUCTION

DELFIA® Tb-N1 DTA Chelate is optimized for the terbium labeling of proteins and peptides for use in dissociation-enhanced time-resolved fluorometric assays. The reagent is the Tb^{3+}-chelate of N1-[p-(3,5-dichlorotriazinyl)benzyl]diethylenetriamine-N^1, N^2, N^3, N^3-tetraacetic acid. The dichlorotriazinyl group reacts with free amino groups on the proteins and peptides, forming a stable, covalent bond.

PACKAGE CONTENTS

1 vial (1 mg, 1.3 µmol) of Tb-N1 DTA Chelate
1 vial (0.5 mL) of 100 nmol/L Terbium Standard

STORAGE

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

REAGENT RECONSTITUTION

Dissolve the chelate in distilled water (e.g. in 100 µL giving 13 mmol/L solution of the chelate) for immediate use. Keep at 0°C (icebath).

If all chelate is not used during the same day, it can be dissolved either in 10 mmol/L sodium succinate (pH 5.0) or in 10 mmol/L sodium acetate (pH 4.8) (pH of the reconstituted chelate should be below 7 for storage purposes). Keep at 0°C (icebath).

RECONSTITUTED STABILITY

The chelate reconstituted in water should be used the same day.

For a long-term storage at -20°C, the chelate should be dissolved in succinate or acetate buffer and aliquoted.

WARNINGS AND PRECAUTIONS

This labeling reagent is intended for research use only.

The handling of concentrated Tb^{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 PROTEINS

Tb-N1 DTA chelate has a dichlorotriazinyl group as a reactive arm. Dichlorotriazinyl group reacts with primary aliphatic amino groups at alkaline pH.

1. Parameters of labeling reaction

   Parameters of labeling reaction include protein concentration, pH, temperature, reaction time and molar excess of chelate over protein.

2. Labeling

   The recommended reaction conditions for labeling of proteins are pH 9 - 9.3, +4°C and overnight incubation. Under these conditions, the following calculations are valid for labeling of a protein with an isoelectric point (pI) between 4 and 7.

Protein concentration (mg/mL)	Percentage of chelate reacted
5	50 %
2.5	25 %
1	10 %

Table 1. The effect of protein concentration on the percentage of Tb-N1 DTA chelate reacting with the protein.

For example, if a protein (pI around 6, molecular weight 160 000) is reacted at a concentration of 5 mg/mL under the conditions described above, a 16-fold molar excess of chelate over protein would give a labeling degree of about 8 Tb-N1 DTA chelates per protein.

If the protein to be labeled is not stable in the labeling conditions (+4°C, pH 9 - 9.3, overnight incubation), it is possible to run a 4-hour reaction (+4°C, pH 9 - 9.3) by increasing the molar excess of chelate over protein. A suitable amount of chelate is three times higher for the 4-hour reaction than for the overnight reaction. For example, if a protein (5 mg/mL in the labeling reaction) requires 16-fold molar excess of chelate during overnight reaction for the introduction of 8 chelates per protein, 48-fold molar excess of Tb-N1 DTA chelate is needed to obtain the same label incorporation during a 4-hour reaction.

Suitable number of Tb-N1 DTA chelates coupled to a protein depends on the molecular weight (MW). When the MW of a protein is higher than 100 000, 4 - 15 chelates per protein is a good labeling yield. For proteins with a MW in the range of 30 000 - 100 000 the preferred number of coupled chelates is 2 - 10. Proteins with a MW less than 30 000 should be labeled with 1 - 3 chelates. The given values may be higher for basic proteins (pI between 8 and 10).

LABELING OF PEPTIDES

Peptides (size up to about 40 amino acids) are labeled like proteins except that the molar excess of chelate over peptide is lower than in protein labeling. Recommended molar excess of chelate over peptide is 3 - 4 (peptide concentration 5 - 20 mg/mL), 3 - 5 (peptide concentration 2.5 - 5 mg/mL) or 5 - 15 (peptide concentration 1 - 2.5 mg/mL). Labeling is usually performed at +4°C but, if the peptide to be labeled is very stable, it can be labeled at room temperature (+20 - +25°C). Suitable number of chelates coupled to a peptide is 1 - 2 depending on the peptide.

LABELING PROCEDURE

The protein or peptide to be labeled must not be stabilized with a protein (e.g. bovine serum albumin (BSA), casein or gelatin).

1. Pretreatment

   If the buffer including the protein or peptide to be labeled contains primary amines (e.g. Tris, ammonium ions), sulfhydryl groups (e.g. mercaptoethanol) or sodium azide, a pretreatment step is necessary. The above mentioned compounds interfere with labeling. Suitable methods for removing interfering compounds include gel filtration, dialysis and reverse phase HPLC (RP-HPLC).

2. Concentrating protein and peptide

   If a protein is too dilute (less than 1 mg/mL) or it is preferable to use less chelate to facilitate purification after labeling, a concentration step is necessary. Suitable concentrators are e.g. Centricon and Centriprep concentrators[1].

   If the concentration of a peptide is too low for an efficient labeling reaction (less than 1 mg/mL), a vacuum centrifuge can be used to concentrate a peptide solution.

3. Reconstitution of chelate

   After calculating the amount of chelate needed in the reaction, Tb-N1 DTA chelate is dissolved either in water (for immediate use only) or in 10 mmol/L sodium succinate pH 5.0 or in 10 mmol/L sodium acetate pH 4.8 (in case some of the chelate will be stored for future purposes). Suitable concentration for reconstituted Tb-N1 DTA is 5 - 30 mmol/L (1 mg of Tb-N1 DTA is 1.3 µmol). For example, dissolving 1 mg of Tb-N1 DTA chelate in 200 µL gives a concentration of 6.5 mmol/L. After dissolving the chelate is kept on ice for immediate use.

[1] Centricon and Centriprep are registered trademarks of Millipore Corp.

4. Labeling

If the protein or peptide is already in a labeling buffer (50 - 100 mmol/L sodium carbonate, pH 9 - 9.3) after the pretreatment or reconstitution, the calculated amount of chelate is added into the protein (peptide) solution on ice.

If the protein (peptide) is not in a labeling buffer, 1 mol/L sodium carbonate (pH 9 - 9.3) is added to adjust buffer concentration to 50 - 100 mmol/L followed by the calculated amount of reconstituted chelate.

In both cases, pH is checked after adding the chelate by applying a 0.5 µL sample on a pH-paper or pH-stick. It is advisable to check the performance of the pH-paper or pH-stick with 50 - 100 mmol/L sodium carbonate buffer of known pH. A suitable pH-paper is Spezial Indikatorpapier pH 8.2 - 10.0 (Merck Art. No. 9558). If necessary, pH of the reaction mixture is adjusted to 9 – 9.3 using either 0.5 - 1 mol/L HCl or 0.5 - 1 mol/L NaOH.

After adding all necessary components and checking pH, incubate at +4°C overnight (or for 4 hours).

5. Purification

Separation of the labeled protein from unreacted chelate is performed by gel filtration. Elution buffer should be Tris-HCl based, e.g. 50 mmol/L Tris-HCl (pH 7.8) containing 0.9 % NaCl and 0.05 % sodium azide (TSA buffer). Proteins with a molecular weight over 100 000 can be purified using Superdex 200 column or a combination of Sephadex G-50 (e.g. 1 x 10 cm) layered on Sepharose 6B (e.g. 1 x 30 - 40 cm) [2]. Proteins with a MW in the range of 30 000 - 100 000 are best purified using Superdex 75 or Sephadex G-50. Sephadex G-50 is suitable also for purification of proteins with a MW between 10 000 and 30 000.

Peptides having at least about 25 amino acid residues can be purified from the unreacted chelate on Sephadex G-25 or alternatively using reverse phase HPLC. Small peptides (less than 25 amino acid residues) can be purified from the unreacted chelate and at least in some cases also from the unlabelled peptide by using reverse phase HPLC. The labeled peptide is eluted from the column in acetonitrile gradient in 0.02 - 0.1 mol/L triethylammonium acetate (pH 7.5). After collecting the labeled peptide acetonitrile is evaporated. It is advisable to add 50 µL/mL 1 mol/L Tris-HCl (pH 8.5) before evaporation of acetonitrile to make sure that pH stays neutral.

[2] Superdex, Sephadex and Sepharose are trademarks of Amersham Pharmacia Biotech.

Proteins MW above 100 000	Proteins MW 30-100 000	Proteins MW 10-30 000	Proteins and peptides MW 2500-10 000	Peptides MW below 2500 (< 25 aa)
Superdex 200	Superdex 75	Sephadex G-50	Sephadex G-25	RP-HPLC
Sephadex G-50 /Sepharose 6B	Sephadex G-50		RP-HPLC	

Table 2. Recommended columns for purification of proteins and peptides after labeling with Tb-N1 DTA chelate.

There should be dedicated columns for each lanthanide (europium, terbium, samarium, dysprosium) used for labeling. After purification, the gel filtration column should be decontaminated by washing with 10 mmol/L phthalate buffer (pH 4.1) containing 0.01 % N1. Before each run, it is advisable to saturate and further purify the gel filtration column on the previous day by applying concentrated BSA solution of high purity (e.g. 0.5 mL 7.5 % BSA; for suitable BSA see section STORAGE OF LABELLED COMPOUNDS). After adding the BSA the column should be equilibrated overnight. RP-HPLC columns can be washed using the phthalate buffer described above.

CHARACTERIZATION OF LABELED PROTEINS AND PEPTIDES

To determine Tb^{3+} concentration in the labeled proteins (peptides), the labeled protein (peptide) is diluted in DELFIA Enhancement Solution (prod. no. 1244-105) and mixed gently. Wait for 5 minutes to allow Tb to dissociate and then add 1/5 of the total volume (e.g. 0.2 mL into 0.8 mL to give a total volume 1 mL; final dilution 1:10 000) DELFIA Enhancer (prod. no. C500-100). After mixing and incubating for 2 minutes measure in a time-resolved fluorometer against 100 nmol/L Tb standard diluted 1:100 in the same way as the sample (1 nmol/L Tb in Enhancement Solution plus DELFIA Enhancer in a clear 96-well plate, 250 µL per well, gives about 500 000 cps in 1420 VICTOR™ Multilabel Counter).

The protein (peptide) concentration can be measured with a suitable method (e.g. Lowry) or calculated from the absorbance at 280 nm. Absorptivity of the reacted Tb-N1 DTA chelate is 13800 at 280 nm (1 µmol/L reacted chelate gives absorbance of 0.0138 at 280 nm).

FILTRATION

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

VICTOR is a trademark of Wallac Oy.

STORAGE OF LABELED COMPOUNDS

To ensure stability, the labeled proteins and peptides should be stored at a high concentration and in the absence of chelators or competing metals in the buffer. A concentrated solution (0.1 mg/mL or higher) can be stored without any stabilizer. With lower concentrations, the stability can be increased by adding purified BSA (available as a specialty product from PerkinElmer Life Sciences, prod. no. CR84-100 Stabilizer) to a final concentration of 0.1 %. Temperature during storage is determined by the stability of the protein. Suitable temperatures are e.g. +4°C, -20°C and -70°C.

USE OF LABELED PROTEINS

The amount of proteins, incubation time, temperature and the buffers used must be optimized for each particular analyte. As a general rule about 5 - 100 ng of the labeled proteins per tube or well can be used. The DELFIA Assay Buffer (product no. 1244-106) is optimal for most assays. It contains NaCl, Tris-HCl, bovine serum albumin (BSA), bovine gamma globulins, Tween 40, diethylenetriaminepentaacetic acid (DTPA), < 0.1 % NaN_3 and an inert red dye. If this assay buffer cannot be used, it is recommended to use a Tris-HCl buffer containing 20 µmol/L EDTA or DTPA to keep the fluorescence background low.

The labeled protein as such is practically non-fluorescent. After binding assay DELFIA Enhancement Solution dissociates Tb ions from labeled protein into solution. For detection of Tb-labeled proteins first add 200 µL of Enhancement Solution per well and shake **slowly** for 5 minutes. Then add 50 µL of DELFIA Enhancer to each well and shake **slowly** for 5 minutes before measuring with the time-resolved fluorometer (1420 VICTOR or 1234 DELFIA Research Fluorometer).

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.

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