TECHNICAL DATA SHEET

LANCE®

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

Eu-W8044 DTA Chelate & Europium Standard

Product Number: AD0020

INTRODUCTION:

Fluorescent dichlorotriazinyl-activated (DTA-activated) Eu-W8044 chelate is optimized for labelling proteins and peptides containing at least one primary aliphatic amino group. The labelled compound can be used in LANCE™ homogeneous time-resolved fluorescence resonance energy transfer (TR-FRET) assays and fluorescence quenching assays
(TR-FQA).

PACKAGE CONTENTS:

1 vial (2 mg, 2.2 µmol) of Eu-W8044 DTA Chelate
1 vial (0.5 mL) of 100 nmol/L Europium Standard

STORAGE:

Store the chelate 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 22 mmol/L solution of the chelate) for immediate use. Keep at 0°C (ice bath).

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 (ice bath).

RECONSTITUTED STABILITY:

The chelate reconstituted in water should be used the same day.
For longterm storage at -20°C, the chelate should be dissolved in succinate or acetate buffer and aliquoted.

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 PROTEINS:

Eu-W8044 DTA chelate has a dichlorotriazinyl group as a reactive arm. Dichlorotriazinyl group reacts with primary aliphatic amino groups at alkaline pH.

1. Parameters of labelling reaction

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

2. Labelling

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

Protein concentration (mg/mL)	Percentage of chelate reacted
5	60 %
2.5	30 %
1	12 %

Table 1. The effect of protein concentration on the percentage of Eu-W8044 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 10-fold molar excess of chelate over protein would give a labelling degree of about 6 Eu-W8044 chelates per protein.

If the protein to be labelled is not stable in the labelling 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 labelling reaction) requires 10-fold molar excess of chelate during overnight reaction for the introduction of 6 chelates per protein, 30-fold molar excess of W8044 is needed to obtain the same label incorporation during a 4-hour reaction.

Suitable number of W8044 chelates coupled to a protein depends on the molecular weight (MW). When the MW of a protein is higher than 100 000, 4 - 10 chelates per protein is a good labelling yield. For proteins with a MW in the range of 30 000 -

70 000, the preferred number of coupled chelates is 2 - 6. Proteins with a MW less than 30 000 should be labelled with 1 - 2 chelates. The given values may be higher for basic proteins (pI between 8 and 10).

LABELLING OF PEPTIDES:

Peptides (size up to about 40 amino acids) are labelled like proteins except that the molar excess of chelate over peptide is lower than in protein labelling. Recommended molar excess of chelate over peptide is 3 - 4 (peptide concentration 5 - 20 mg/mL), 4 - 6 (peptide concentration 2.5 - 5 mg/mL) or 6 - 12 (peptide concentration 1 - 2.5 mg/mL). Labelling is usually performed at +4°C but, if the peptide to be labelled is very stable, it can be labelled at room temperature (+20 - +25°C). Suitable number of chelates coupled to a peptide is 1 - 2 depending on the peptide.

LABELLING PROCEDURE:

The protein or peptide to be labelled 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 labelled 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 labelling. 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 labelling, 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 labelling reaction (less than 1 mg/mL), a vacuum centrifuge can be used to concentrate the peptide solution.

3. Reconstitution of chelate

   After calculating the molar excess of chelate needed in the reaction, Eu-W8044 DTA chelate is dissolved either in distilled water (for immediate use only) or in 10 mmol/L sodium succinate pH 5.0 or 10 mmol/L sodium acetate pH 4.8 (in case some of the chelate will be stored at -20°C for future purposes). Suitable concentration for reconstituted Eu-W8044 DTA chelate is 2.5 - 20 mmol/L (2 mg of Eu-W8044 DTA is 2.2 µmol). For example, dissolving 2 mg of Eu-W8044 DTA chelate in 100 µL water gives a concentration of 22 mmol/L. After dissolving the chelate it is kept on ice for immediate use.

4. Labelling

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

If the protein or peptide is already in a labelling 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 labelling 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 W8044.

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 the pH, incubate at +4°C overnight (or for 4 hours).

5. Purification

Separation of the labelled protein from unreacted chelate is performed by gel filtration or possibly by a combination of a concentration step (e.g. with Centricon concentrators) and gel filtration. Using a concentrator removes some of the unreacted chelate making purification more efficient. Concentration step is started by adding TSA buffer (see below) up to e.g. 2 mL, and then the reaction mixture is concentrated to
0.2 – 0.5 mL. The concentration step may be repeated.

Purification 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 DNA Grade (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 DNA Grade. Sephadex G-50 DNA Grade is suitable also for purification of proteins with a MW between 15 000 and
30 000.

Peptides can be purified from the unreacted chelate using reverse phase HPLC. The labelled peptide is eluted from the column in acetonitrile gradient in 0.02 - 0.1 mol/L triethylammonium acetate (pH 7.5). After collecting the labelled 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.

Proteins MW above 100 000	Proteins MW 30-100 000	Proteins MW 15-30 000	Proteins and peptides MW 2500-15 000	Peptides MW below 2500 (< 25 aa)
Superdex 200	Superdex 75	Sephadex G-50 DNA Grade		RP-HPLC
Sephadex G-50 /Sepharose 6B	Sephadex G-50 DNA Grade		RP-HPLC	

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

Table 2. Recommended columns for purification of proteins and peptides after labelling with Eu-W8044 DTA chelate.

There should be dedicated columns for each lanthanide (europium, terbium, samarium, dysprosium) used for labelling. After purification, the gel filtration column should be decontaminated by washing with 10 mmol/L phthalate buffer (pH 4.1) containing 0.01 % DTPA. 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. However, as it may be difficult to purify a gel filtration column after
Eu-W8044 DTA labelling, it is recommended to use a gel filtration column only once. RP-HPLC columns can be washed using the phthalate buffer described above.

CHARACTERIZATION OF LABELLED PROTEINS AND PEPTIDES:

Determination of the Eu^{3+} concentration in the Eu-W8044-labelled proteins (or peptides) can be performed as follows. First the labelled protein (peptide) is diluted in 0.1 mol/L HCl (e.g. 1:100) and incubated at room temperature for 10 minutes. The final dilution is performed in DELFIA Enhancement Solution (prod. no. 1244-105) and the dilution factor has to be at least 1:100 to dilute out the effect of HCl on the pH of Enhancement Solution. Eu fluorescence of the sample is measured against 100 nmol/L Eu standard (supplied with the chelate) diluted 1:100 in Enhancement Solution (1 nmol/L Eu in Enhancement Solution in a clear 96-well plate, 200 µL per well, gives about 1 000 000 counts in 1234 DELFIA Research Fluorometer or 1420 VICTOR™ Multilabel Counter).

Protein (peptide) concentration can be measured with a suitable method or calculated from absorbance at 280 nm and 335 nm. The contribution of the reacted Eu-W8044 DTA chelate to absorbance at 280 nm is $0.8 \times A_{335}$.

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 W8044-labelled 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 Wallac Oy, 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.

VICTOR is a trademark of PerkinElmer.

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 exept 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 Upgraged**	**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

When Eu-W8044-labelled compounds are used in LANCE homogeneous assays, extending cycle time in the measurement to 2000 µs may improve sensitivity.

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.

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