

DELFLIA[®] EuTDA Cytotoxicity Assay Reagents AD0116

Development grade

INTENDED USE

This product is intended for labelling of cells to be used in short term cytotoxicity tests performed utilizing time-resolved fluorometry in the detection.

INTRODUCTION

Each Eu-ligand release assay product contains

- BATDA Reagent for labelling of cells
- Lysis Buffer for lysis of cells to quantitate maximal release
- Europium Solution and microtitration plates for measuring Eu signal

The procedure is based on loading target cells with a fluorescence enhancing ligand (BATDA, bis (acetoxymethyl) 2,2':6',2"- terpyridine- 6,6"- dicarboxylate). The hydrophobic ligand penetrates the membrane quickly. Within the cell the ester bonds are hydrolysed to form a hydrophilic ligand (TDA, i.e. 2,2':6',2"- terpyridine-6,6"- dicarboxylic acid) which no longer pass the membrane. After cytolysis the ligand is released and introduced to the DELFLIA[®] Europium Solution. The europium and the ligand form a highly fluorescent and stable chelate (EuTDA). The measured signal correlates directly with the amount of lysed cells.

PACKAGE CONTENTS

The reagents are sufficient for at least 10 one-plate assays when the suggested labelling protocol is used.

Component	Quantity	Shelf life and storage
DELFLIA BATDA Reagent	1 vial, 50 µL	+2 - +8°C until expiry date stated on the vial label.
Ready for use reagent dissolved in dimethylsulphoxide (DMSO).		
DELFLIA Lysis Buffer	1 vial, 0.5 mL	+2 - +8°C until expiry date stated on the vial label.
Ready for use solution containing digitonin (1 mg/mL) and 19 % dimethylsulphoxide (DMSO).		

DELFLIA Europium Solution	1 bottle, 200 mL	+2 - +8°C until expiry date stated on the bottle label.
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Ready for use europium solution based on acetate buffer (pH 4).

DELFLIA Microtitration Plates	10 plates	+2 - +8°C
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MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE PRODUCT

1. Tissue culture plates (round-bottom)
2. Suitable wash solution for the cell line, balanced salt solution like PBS or the cell culture medium
3. Cell culture medium, e.g. RPMI 1640 (Gibco)
4. Time-resolved fluorometer: 1420 VICTOR™, 1420 VICTOR² or 1234 DELFLIA Research Fluorometer
5. Pipette for dispensing the Europium Solution - Eppendorf Multipipette (prod. no. 1296-014) with 5 mL Combitips (prod. no. 1296-016), or alternatively the DELFLIA Plate Dispense (prod. no. 1296-041)
6. Automatic shaker - DELFLIA Plateshake (prod. no. 1296-001/002 or 1296-003/004)

WARNINGS AND PRECAUTIONS

The Eu-ligand release assay reagents are intended for *in vitro* research use only.

The protocols given in this insert are only guidelines and they may need optimization depending on the cell line used.

NOTE: The DELFLIA BATDA Reagent is dissolved in dimethylsulphoxide (DMSO) and the Lysis Buffer contains digitonin and dimethylsulphoxide (DMSO).

DMSO is absorbed through skin, is irritating to eyes and skin, causes nausea, vomiting and tiredness. Use gloves approved for chemicals. If a risk of splashing occurs, wear safety goggles. In case of contact with eyes rinse immediately with plenty of water and seek medical advice. After contact with skin wash immediately with plenty of water. Handling in fume cupboard is recommended.

Digitonin is toxic by inhalation, in contact with skin and if swallowed. In contact with eyes or skin rinse immediately with plenty of water and seek medical advice in case of splashes in eyes.

Discard as hazardous waste, in accordance with local regulations.

PROCEDURAL NOTES

Take care that water is not introduced to the fluorescence enhancing ligand (DELFIATM BATDA Reagent) vial when pipetting the reagent to avoid hydrolysis of the ligand. Make sure that the reagent is thawed and mixed before use.

Europium solution contains very high europium levels. Thus, the handling should be well separated from BATDA reagent handling. Due to the very low detection limit of europium (10^{-18} mol), a dedicated pipette for Europium Solution is required to prevent contamination problems. The contamination of BATDA Reagent will result in high fluorescent background. This should be kept in mind also with other DELFIA assays performed in the laboratory.

Depending on the cell line the following parameters may have to be adjusted before labelling:

Labelling temperature:

+4 - +37 °C, use the temperature your cell line stands best. High temperature correlates to faster loading and may be more gentle to sensitive cell lines.

Labelling time:

5 - 30 minutes, usually a very sensitive cell line should not be labelled longer than 5 - 10 minutes. Label until you get a maximal signal higher than 15000 - 20000, avoid loading for too long.

Labelling concentration:

Sometimes it may be necessary to increase the amount of BATDA to achieve sufficiently high fluorescence. However, it is recommended to use as low concentrations as possible to avoid unnecessary washing. Accumulation of large amounts of the hydrolysis product formaldehyde in target cells may be toxic to cells.

Wash steps:

When washing the wells, suspend the cells very carefully and try to wash fast. If necessary, add 1 - 10 mmol/L Probenecid (Sigma P8761) or Sulfapyrazole (Sigma S9509) into the wash solution to lower the spontaneous release.

The amount of cells per well in an assay is normally around 5000 - 10000. The assay incubation time should not exceed 4 hours in order to get acceptable level of spontaneous release. For longer assay times the Eu-DTPA method can be used (see Wallac Application Note number 1234-964).

PREPARATION OF REAGENTS

1. Heat up the Lysis Buffer on water bath (+37°C) prior to use. Digitonin in the Lysis Buffer may precipitate during storage. In case of a white precipitate it is preferable to warm the Lysis Buffer to 50 – 60°C to completely dissolve digitonin. Should precipitation occur, make sure that it is dissolved before use.

2. Let the reagents reach room temperature (+20 - +25°C) before use.
3. Check that the DELFIA BATDA Reagent is thoroughly thawed and mixed before use.

LABELLING PROTOCOL

1. Wash the cells once with a balanced salt solution (eg. PBS) or medium.
2. Adjust the number of cells to about 1×10^6 cells/mL with the culture medium. Add 2 - 4 mL of cells to 5 μ L of the fluorescence enhancing ligand. Incubate for 20 - 30 minutes at +37°C.
3. Spin down the cells and resuspend in wash buffer.
4. Wash the cells 3 - 5 times. Resuspend the pellet carefully. Avoid contamination from one wash step to the following.
5. After the final wash resuspend the pellet in culture medium and adjust to about 5×10^4 cells/mL.

CYTOTOXICITY ASSAY PROTOCOL

1. Pipette 100 μ L of loaded target cells (5000 cells) to a round-bottom sterile plate.
2. Add 100 μ L of effector cells of varying cell concentrations. Effector to target ratio ranges from 6:1 to 100:1 are commonly used for natural killer cells. Remember to set up wells for background, spontaneous release and the maximum release measurements (see below for the definition).
3. Incubate for 2 hours in a humidified 5 % CO₂ atmosphere at +37°C.
4. Centrifuge for 5 minutes at 500x g.
5. Transfer 20 μ L of the supernatant to a flat-bottom plate (included in the kit).
6. Add 200 μ L of Europium Solution.
7. Incubate for 15 minutes at room temperature using the DELFIA Plateshake.
8. Measure the fluorescence in the time-resolved fluorometer.

Definition of samples

Background: Take an aliquot of the loaded target cells and centrifuge it immediately after dilution. Do not incubate the cells. Pipette 100 μ L of the supernatant into the wells and add 100 μ L of the cell culture medium. Transfer a 20 μ L aliquot to the flat-bottom plate and add 200 μ L of the DELFIA Europium Solution, shake for 15 minutes and measure the fluorescence.

Spontaneous release: Incubate the target cells (100 µL) with 100 µL of cell culture medium instead of effector cells during the assay. Centrifuge and transfer 20 µL of the supernatant to the flat-bottom plate and add 200 µL of the DELFIA Europium Solution, shake for 15 minutes and measure the fluorescence.

Maximum release: Incubate the target cells (100 µL) with 100 µL of cell culture medium supplemented with 10 µL of Lysis Buffer. Centrifuge and transfer 20 µL of the supernatant to the flat-bottom plate and add 200 µL of the DELFIA Europium Solution, shake for 15 minutes and measure the fluorescence.

Formulas for calculating

$$\% \text{ Specific release} = \frac{\text{Experimental release (counts)} - \text{Spontaneous release (counts)}}{\text{Maximum release (counts)} - \text{Spontaneous release (counts)}} \times 100$$

$$\% \text{ Spontaneous release} = \frac{\text{Spontaneous release (counts)} - \text{background (counts)}}{\text{Maximum release (counts)} - \text{background (counts)}} \times 100$$

WARRANTY

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