TECHNICAL DATA SHEET

Delfia® Research Reagents

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

EuTDA Cytotoxicity Reagents

Product No.: AD0116

Lot No.: 684-981-A

Material Provided

Format: 960 assay points

Kit Contents:

Reagent	Item Number	Quantity
BATDA Reagent	C136-100	50 µL
Lysis Buffer	AD0116-A	0.5 mL
Europium Solution	AD0116-B	200 mL
Microtitration Strips, uncoated	AD0116-C	10 plates

Expiry Date: **August 15, 2012**

Product Information

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

Storage Conditions: Store at 4°C

Quality Control (Delfia Cytotoxicity Assay)

Max release: 37396

Spontaneous release: 17160

Background: 8380

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Reagents

DELFIA BATDA Reagent, 50 µL

Ready-for-use reagent dissolved in dimethylsulphoxide (DMSO).

Note: 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.

DELFIA Lysis Buffer, 0.5 mL

Ready-for-use solution containing 0.03 % digitonin and 19 % dimethylsulphoxide (DMSO).

Note: 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. Read the warning above before handling DMSO.

DELFIA Europium Solution, 200 mL

Ready-for-use europium solution based on acetate buffer (pH 4).

DELFIA Microtitration Plates, 10 plates

Store between 2 and 25°C

Materials required but not supplied with the product:

1. 96-well plates (V-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: VICTOR™, ViewLux™, EnVision™ or Fusion™
5. Pipette for dispensing the Europium Solution - Eppendorf Multipette (prod. no. 1296-014) with 5 mL Combitips (prod. no. 1296-016), or alternatively the DELFIA Plate Dispense (prod. no. 1296-041)
6. Automatic shaker - DELFIA Plateshake (prod. no. 1296-003/004)

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Recommended Assay Conditions

Procedural Notes:

Take care that water is not introduced to the fluorescence enhancing ligand (DELFIA BATDA Reagent) vial when pipetting the reagent to avoid hydrolysis of the ligand. Make sure that the reagent is thawed and mixed before use. The reagent should be frozen at +4°C. If the reagent is melted, it may be inactive. Repeated opening of the vial may cause hydrolysis of the reagent.

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 (10E-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 loading:

Loading temperature:

From 4 to 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.

Loading time:

5 - 30 minutes, usually a very sensitive cell line should not be loaded longer than 5 - 10 minutes. The loading time needs to be optimized for each cell line and for each type of experiment separately.

Loading 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 cells, suspend the cells very carefully and try to wash fast. If necessary, add 1 - 10 mmol/L Probenecid (Sigma P8761) or Sulfinpyrazone (Sigma S9509) into the wash solution to lower the spontaneous release. Check that the pH of the wash buffer is not affected by addition of probenecid or sulfinpyrazone.

Assay optimizations:

The amount of target cells per well in an assay is normally around 5000 - 10000.

The assay incubation time in step 4 ("CYTOTOXICITY ASSAY PROTOCOL") should not exceed 4 hours in order to get acceptable level of spontaneous release.

The monitoring of the assay kinetics is possible in step 6 ("CYTOTOXICITY ASSAY PROTOCOL"). The samples and the controls (20 µL) can be transferred at the time points of interest (5 min. - 4 h).

Preparation of Reagents:

1. Heat up the Lysis Buffer on a water bath (37°C) prior to use. Digitonin in the Lysis Buffer may precipitate during storage. Should precipitation occur, warm the Lysis Buffer to 50 - 60°C to completely dissolve the digitonin.

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.

Loading Protocol:

1. Wash the cells once with a balanced salt solution (eg. PBS) or medium.

2. Adjust the number of cells to about 1 x 10^6 cells/mL with the culture medium. Add 2 - 4 mL of cells to 5 µL of the fluorescence enhancing ligand. Incubate for 5 - 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 x 10^4 cells/mL.

NOTE: Do not incubate or leave the cells waiting at this point, proceed immediately to the next step in the assay.

Cytotoxicity Assay Protocol:

1. Set up wells for detection of background, spontaneous release and maximum release (see below for the definition).

2. Pipette 100 µL of loaded target cells (5000 cells) to a V-bottom plate.

3. Add 100 µL 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.

4. Incubate for 2 hours in a humidified 5 % CO_2 atmosphere at 37°C.

5. Centrifugation for 5 minutes at 500x g is recommended. This is not compulsory if in step 6 the supernatant is transferred carefully (avoiding cells) from the V-bottom plate.

6. Transfer 20 µL of the supernatant to a flat-bottom plate (included in the kit).

7. Add 200 µL of Europium Solution.

8. Incubate for 15 minutes at room temperature using the DELFIA Plateshake.

9. Measure the fluorescence in the time-resolved fluorometer.

Definitions of background, spontaneous release and maximum release:

Background (= media without cells): Take an aliquot of the loaded target cells immediately after dilution in culture medium. Do not incubate the cells. Centrifuge the cells down and pipette 100 µL of the supernatant into the wells and add 100 µL of the cell culture medium. Transfer the same way as the samples.

Spontaneous release (= target cells without effector cells): Incubate the target cells (100 µL) with 100 µL of cell culture medium instead of effector cells during the assay. Transfer the same way as the samples.

Maximum release (= lysed target cells): Incubate the target cells (100 µL) with 100 µL of cell culture medium supplemented with 10 µL of Lysis Buffer. Transfer the same way as the samples.

Formulas for calculating

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

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

Note that in some cases the effector cells are more effective for lysing the target cells than is the Lysis Buffer. In that case, the experimental release may be higher than the maximum release and these formulas can not be applied.

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