DELFIA® Cell Proliferation kit AD0200

For Research Use Only

INTENDED USE

This DELFIA® Cell Proliferation kit is intended for the rapid and simple assay of cytotoxicity or proliferation of mammalian cells and cell lines in culture (adherent and suspension cells). The kit can be used for the direct assessment of cell numbers, and also for assaying cytotoxic effects on cultured cells as an endpoint measurement. DELFIA Cell Proliferation kit is a non-isotopic immunoassay based on the measurement of 5-bromo-2’-deoxyuridine (BrdU) incorporation during DNA synthesis in proliferating cells.

INTRODUCTION

Cell proliferation is an important parameter when studying live cell function, especially, when the effect of growth regulatory substances or cytotoxic agents are under study. A number of methods have been developed to measure the proliferation of cells. These methods are based on microscopic detection, incorporation of radioactive precursors, uptake of chromogenic dyes or measurement of metabolic activity of proliferating cells. Since cellular proliferation requires the replication of cellular DNA, methods based on DNA synthesis measurement can be used as an accurate indicator of cell growth. Traditionally, ^{3}H-thymidine has been used to label DNA. A non-isotopic alternative for ^{3}H-thymidine is BrdU, a pyrimidine analog, which can be incorporated into newly synthesized DNA instead of thymidine. BrdU is detected immunochemically. The use of BrdU to measure cell proliferation and the immunochemical detection of BrdU has been described by several investigators (1–5).

PRINCIPLES OF THE ASSAY

The DELFIA Cell Proliferation assay is a time-resolved fluoroimmunoassay based on the incorporation of BrdU into newly synthesized DNA strands of proliferating cells cultured in microplates. Incorporated BrdU is detected using europium labeled monoclonal antibody. To allow antibody detection cells are fixed and DNA denatured using Fix Solution. Unbound antibody is washed away and DELFIA Inducer is added to dissociate europium ions from the labeled antibody into solution, where they form highly fluorescent chelates with components of the DELFIA Inducer (6–7). The fluorescence measured is proportional to the DNA synthesis in the cell population of each well.

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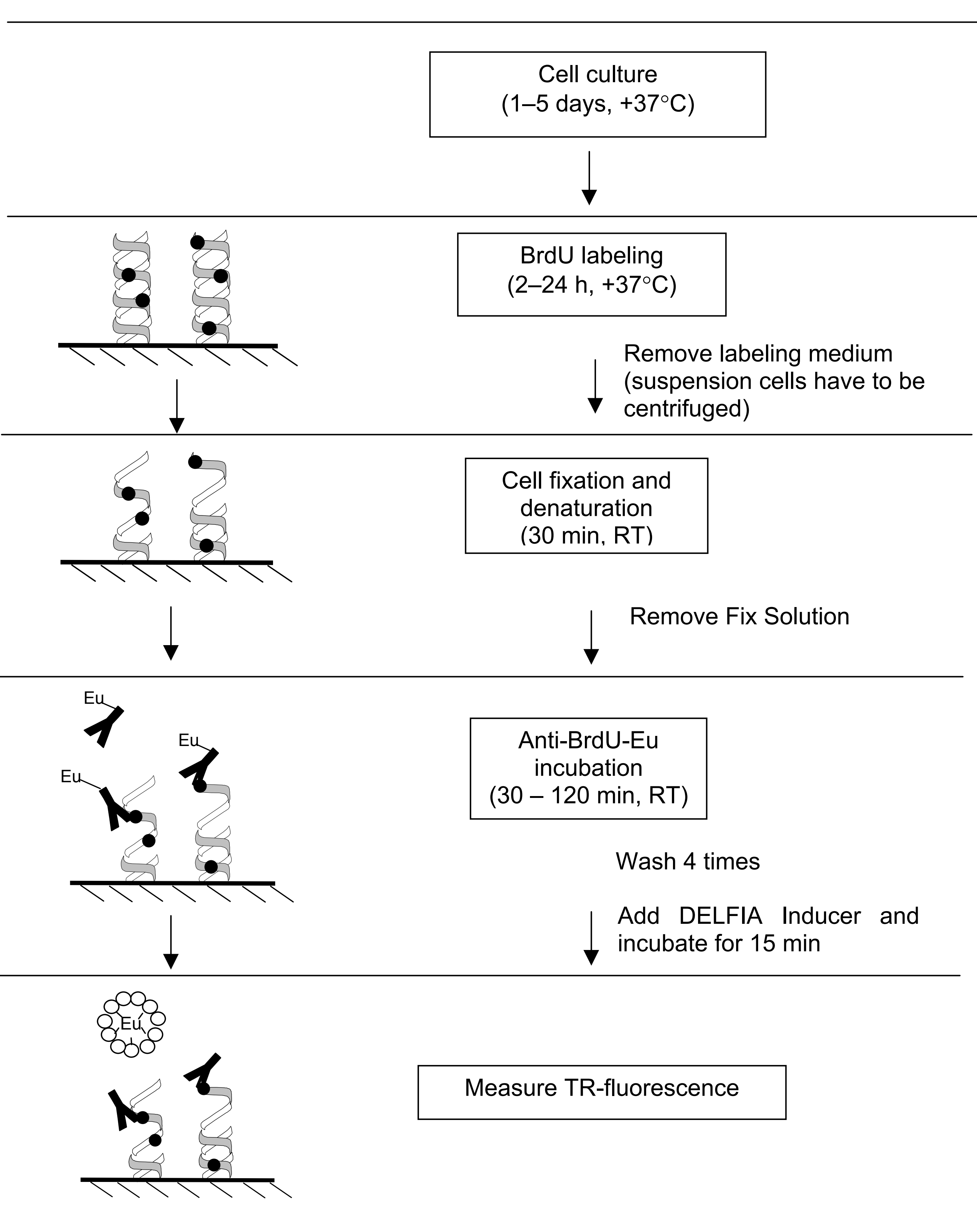
The assay procedure is dependent on the cell line used and exact incubation times have to be optimized for each experimental setup individually. The following assay procedure is appropriate for most applications:



KIT CONTENTS

The reagents are sufficient for 960 assays.

The expiry date of the complete kit is stated on the kit label. Store at +2 - +8°C.

Reagents

<table>
<tr><th>Component</th><th>Quantity</th><th>Shelf life and storage</th></tr>
<tr><td>BrdU Labeling Reagent</td><td>1 vial, 0.3 mL</td><td>+2 - +8°C until expiry date stated on the kit label.
Protect from light.</td></tr>
<tr><td colspan="3">Reagent containing 10 mM 5-bromo-2'-deoxyuridine (1000 x conc.) diluted in phosphate-buffered saline solution (pH 7.4), sterile filtered.</td></tr>
<tr><td>Anti-BrdU-Eu</td><td>1 vial, lyophilized</td><td>+2 - +8°C until expiry date stated on the kit label.</td></tr>
<tr><td colspan="3">The Eu-labeled mouse monoclonal antibody is in Tris-HCl buffered (pH 7.8) salt solution with Dextran T10 and bovine serum albumin.

NOTE: The powder contains sodium azide (< 1 %) as preservative and it is harmful by inhalation, in contact with skin and if swallowed.</td></tr>
<tr><td>Fix Solution</td><td>1 bottle, 175 mL</td><td>+2 - +8°C until expiry date stated on the kit label.</td></tr>
<tr><td colspan="3">Ready-for-use Fix Solution containing ethanol.

NOTE: The solution is flammable.</td></tr>
<tr><td>Wash Concentrate</td><td>1 bottle, 250 mL</td><td>+2 - +8°C until expiry date stated on the bottle label.</td></tr>
<tr><td colspan="3">A 25-fold concentration of Tris-HCl buffered (pH 7.8) salt solution with Tween 20. Contains Germall II[1] as preservative.</td></tr>
<tr><td>Assay Buffer</td><td>1 bottle, 175 mL</td><td>+2 - +8°C until expiry date stated on the bottle label.</td></tr>
<tr><td colspan="3">Ready-for-use Tris-HCl buffered (pH 7.8) salt solution with bovine serum albumin, bovine globulin, Tween 40, an inert red dye, and < 0.1 % sodium azide as preservative.</td></tr>
</table>

[1] Germall is a registered trademark of Sutton Laboratories Inc.

DELFIA Inducer[2]	1 bottle, 250 mL	+2 - +8°C until expiry date stated on the bottle label. Shelf life 2 weeks in dark, at room temperature (+20 - +25°C). **Protect from light.**

Ready-for-use Inducer solution with Triton X-100[3], glycine, hydrochloric acid and chelators.

MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT

The DELFIA system requires the following items, which are available from PerkinElmer Life Sciences or its distributors.

1. White 96-well microplates for tissue culture (clear, flat bottom), e.g. Isoplate™ (prod. no. 1450-517) or ViewPlate® [4](cat. no. 6005181)
2. Pipette for dispensing the Fix Solution and the Eu-labeled anti-BrdU - Eppendorf Multipette (prod. no. 1296-014) with 5 mL Combitips (prod. no. 1296-016)
3. Pipette for dispensing the DELFIA Inducer - Eppendorf Multipette (prod. no. 1296-014) with 5 mL Combitips (prod. no. 1296-016) or alternatively the DELFIA Plate Dispense (prod. no. 1296-041)
4. Automatic washer - DELFIA Platewash (prod. no. 1296-026)
5. Automatic shaker - DELFIA Plateshake (prod. no. 1296-003/004)
6. Time-resolved fluorometer, e.g. 2100 EnVision™ Multilabel Reader

In addition to the DELFIA system the following are required:

- precision pipettes for dispensing microliter volumes and pipettes for dispensing milliliter volumes
- distilled water

WARNINGS AND PRECAUTIONS

This DELFIA Cell Proliferation kit is intended for research use only.

Lyophilized anti-BrdU-Eu contains sodium azide. The powder is harmful by inhalation, in contact with skin and if swallowed.

Reagents contain sodium azide (NaN_3) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

[2] Patent pending.
[3] Triton is a registered trademark of Rohm and Haas Co.
[4] Isoplate and EnVision are trademarks and ViewPlate is a registered trademark of PerkinElmer, Inc.

The Fix Solution is flammable.

Disposal of all waste should be in accordance with local regulations.

PREPARATION OF REAGENTS

Reagent	Reconstituted stability
BrdU Labeling Solution, 100 µM	2 weeks at +2 - +8°C. For long-term storage aliquot and store at -20°C. Protect from light.

Dilute BrdU Labeling Reagent 1 : 100 with sterile culture medium. For one 96-well microplate, e.g. if the cells were cultured in 200 µL culture medium per well, pour 20 µL of BrdU Labeling Reagent into a clean container and add 2 mL of sterile culture medium.

Reagent	Reconstituted stability
Anti-BrdU-Eu stock solution, 50 µg/mL	2 weeks at +2 - +8°C. For long-term storage aliquot and store at -20°C. Repeated freezing and thawing must be avoided.

Reconstitute the lyophilized anti-BrdU-Eu by adding exactly 1.2 mL of distilled water. Mix gently and allow to stabilize 30 minutes before use.

NOTE: The powder contains sodium azide (< 1 %) as preservative and it is harmful by inhalation, in contact with skin and if swallowed. The dissolved anti-BrdU-Eu stock solution contains < 0.1 % sodium azide and is not considered harmful.

Reagent	Reconstituted stability
Anti-BrdU-Eu working solution, 0.5 µg/mL	Prepare only the amount needed within 4 hours.

Dilute reconstituted anti-BrdU-Eu stock solution 1 : 100 with Assay Buffer, e.g. for one 96-well microplate mix 110 µL of reconstituted anti-BrdU-Eu stock solution with 11 mL of Assay Buffer.

We advise the use of disposable plastic container to prepare the anti-BrdU-Eu working solution.

Reagent	Reconstituted stability
Wash solution	2 weeks at +2 - +25°C in a sealed container.

Dilute Wash Concentrate 25-fold with distilled water, e.g. for one 96-well microplate pour 20 mL of Wash Concentrate into a clean container and add 480 mL of distilled water to give a buffered wash solution (pH 7.8).

ASSAY PROCEDURE

All reagents except BrdU Labeling Reagent and anti-BrdU stock solution must be brought to room temperature (+20 - +25°C) before use.

The validity of the experimental setup should be verified in two different ways: **blank** wells (no cells are added to the well, only culture medium + BrdU + anti-BrdU-Eu) provide information about the unspecific binding of BrdU and anti-BrdU-Eu, whereas **background** wells (no BrdU is added to the wells, only cells in culture medium + anti-BrdU-Eu) provide information about the unspecific binding of anti-BrdU-Eu.

1. Place appropriate amount of cells in a 96-well plate (at a final volume of e.g. 200 µL per well) and incubate them with the substance to be tested at +37°C in a humidified 5% CO_2 atmosphere. The incubation period depends on the cell type used. For most experimental approaches, an incubation period of 24–120 hours is appropriate.

2. Label cells with BrdU by adding 20 µL of diluted (100 µM) BrdU Labeling Solution to each well if the cells were cultured in 200 µL of culture medium and re-incubate the cells for additional 2 to 24 hours at +37°C in a humidified 5% CO_2 atmosphere.

   NOTE: The volume of BrdU Labeling Solution to be added depends on the volume of the cell culture, and that the final concentration of BrdU in the wells should be 10 µM.

3. Remove labeling medium thoroughly. Suspension cells have to be centrifuged at 300 x g for 10 minutes before removing the labeling medium.

4. Add 100 µL of Fix Solution to each well and incubate for 30 minutes at room temperature.

5. Remove Fix Solution thoroughly from the wells either by inverting the plate and shaking it, or by aspiration.

6. Add 100 µL Anti-BrdU-Eu working solution (0.5 µg/mL) to each well and incubate for 30–120 minutes at room temperature.

7. Wash 4 times using the DELFIA Platewash with approximately 300 µL of wash solution per well. See point 3 in the section "PROCEDURAL NOTES".

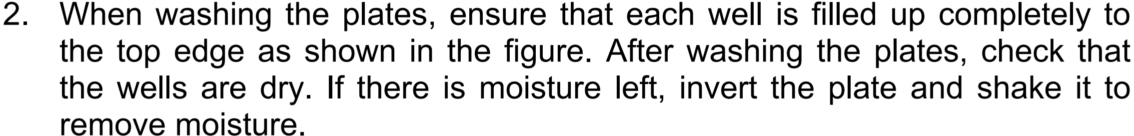
8. Add 200 µL DELFIA Inducer directly from the reagent bottle to each well using the DELFIA Plate Dispense or Eppendorf Multipette. **When using the DELFIA Plate Dispense or any other automated dispensing system, make sure that the tubing is flushed with DELFIA Inducer before dispensing into the well.** When using the Eppendorf Multipette flush the Combitip once with DELFIA Inducer (to waste). Refill the Combitip and discard the first aliquot. Avoid touching the edge of the well or its contents.

9. Shake the plate on the DELFIA Plateshake at room temperature for 15 minutes (slow shaking). When using a different shaker, check that the liquid is moving constantly. The fluorescence is stable for several hours if evaporation is prevented. However, we recommend measurement within 1 hour as external factors may cause a decrease in signal with time, although this is extremely rare.

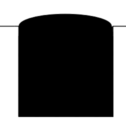
10. Measure the Eu-fluorescence in a time-resolved fluorometer.

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the DELFIA kit. The reagents supplied with this kit are intended for use as an integral unit. Do not mix identical reagents from kits having different lot numbers. Do not use kit reagents after the expiry date printed on the kit label.



2. When washing the plates, ensure that each well is filled up completely to the top edge as shown in the figure. After washing the plates, check that the wells are dry. If there is moisture left, invert the plate and shake it to remove moisture.



   For detailed information on the cleaning and maintenance of the washing device, please refer to the DELFIA Platewash manual.

3. The avoidance of europium contamination and resulting high fluorescent background demands high standard pipetting and washing techniques.

   The DELFIA Inducer should be dispensed using the DELFIA Plate Dispense or the Eppendorf Multipette after the Combitip has been first flushed with DELFIA Inducer. The same Combitip must not be used for pipetting any other reagent.

   When using the DELFIA Plate Dispense, please refer to the manual.

ANALYTICAL PERFORMANCE CHARACTERISTICS

For most experimental setups 100–10 000 cells per well are sufficient. When working with primary lymphocytes 10 000–300 000 cells per well should be used. Optimal amount of cells per well depends on the cell type used and the incubation times applied for the assay.

Figure 1 shows typical data for measuring cell number. Suspension cells (Jurkat) were titrated in the microplate at the numbers per well indicated in the figure. After 4 hours incubation with BrdU, the incorporation was detected as described in the Assay Procedure. There is a linear relationship (R^2 = 0.99) between Jurkat cells and Eu fluorescence up to 500 000 cells per well.

Figure 1

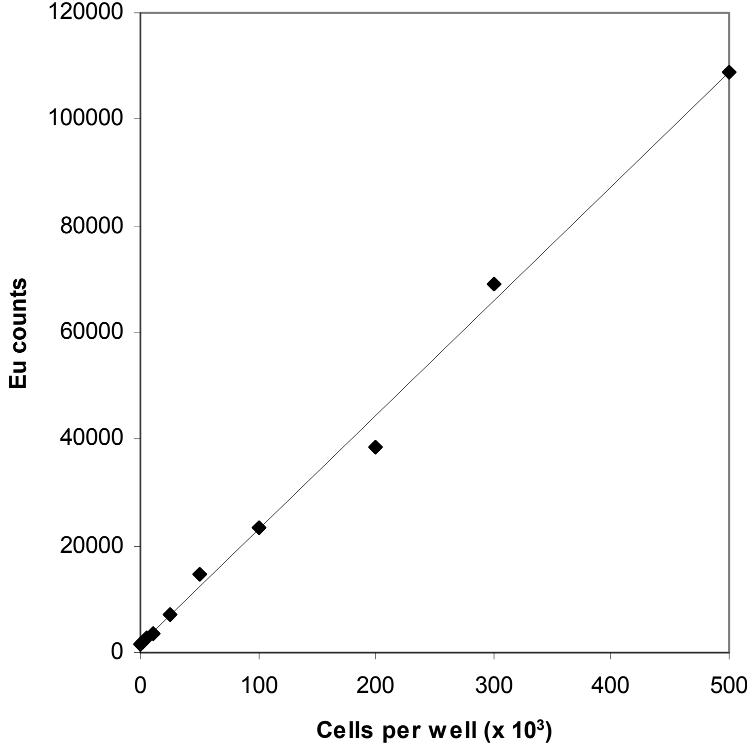
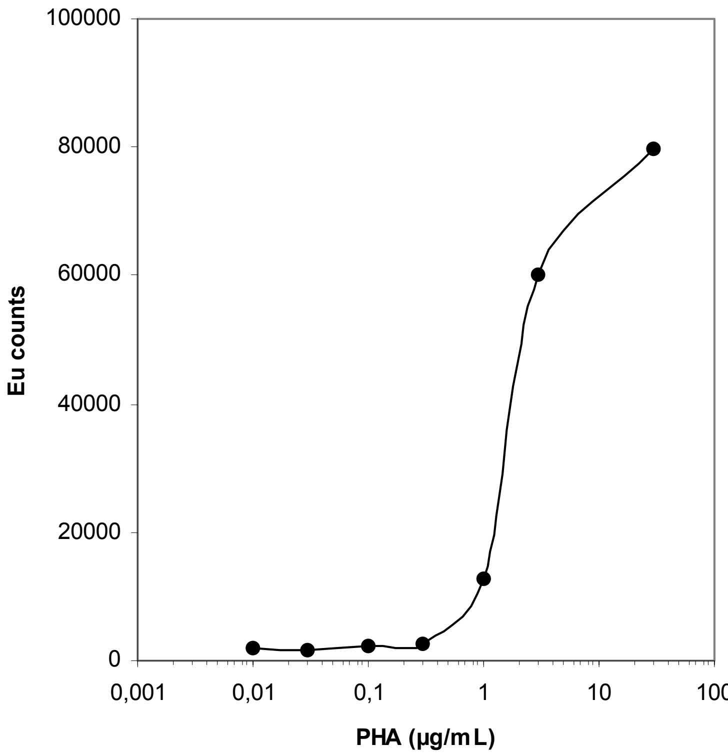


Figure 2 shows results of stimulation assay using human peripheral blood lymphocytes. The cells were isolated by density gradient centrifugation and cultured in microplates (150 000 cells per well) for 48 hours in the presence of various concentrations of phytohaemagglutinin (PHA). After 4 hours incubation with BrdU, the incorporation was determined as described in the Assay Procedure.

Figure 2



WARRANTY

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All information supplied with the product and technical assistance given is believed to be accurate, but it remains the responsibility of the investigator to confirm all technical aspects of the application. We appreciate receiving any additions, corrections, or updates to information supplied to the customer.

REFERENCES

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DELFIA® Cell Proliferation kit

Preparation of reagents

Prepare BrdU Labeling Solution		20 µL BrdU Labeling Reagent + 2.0 mL sterile culture medium per plate (when cells are cultured in 200 µL)
Reconstitute Anti-BrdU-Eu stock solution		Add 1.2 mL distilled water, 30 min.
Prepare Anti-BrdU-Eu working solution		110 µL Anti-BrdU-Eu stock solution + 11 mL Assay Buffer per plate
Prepare wash solution		Dilute 1 : 25 with distilled water

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DELFIA® Cell Proliferation kit

Summary Sheet for Assay Procedure

Step		
Culture cells + test substance (no cells in blank wells)		100 or 200 µL
Add BrdU Labeling Solution (except background wells)		10 µL or 20 µL
Incubate		2–24 h at +37°C
Remove labeling medium		Adherent cells: invert and shake the plate or aspirate. Suspension cells: centrifuge at 300 x g for 10 min.
Add Fix Solution		100 µL
Incubate		30 min. at RT
Remove Fix Solution		Invert and shake the plate or aspirate
Add Anti-BrdU-Eu working solution		100 µL
Incubate		30–120 min. at RT
Wash		x 4
Add DELFIA Inducer		200 µL, 15 min. slow shaking
Measure fluorescence		Eu-filter

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