MANUAL

Cell Proliferation Kit

Product number: AD0200

Research Use Only. Not for use in diagnostic procedures.

Material Provided

Format: 960 assay points

Kit Contents:

Reagent	Volume
BrdU Labeling Reagent	300 µL
Anti-BrdU-Eu	600 µL
Fix Solution	175 mL
Wash Concentrate	250 mL
Assay Buffer	175 mL
Delfia Inducer	250 mL

Expiry Date: See kit box label for expiration date

Product Information

Intended Use: This DELFIA® Cell Proliferation kit is intended for the rapid and simple assay of cytotoxicity or proliferation of 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.

Storage Conditions: Store at 4°C

Preparation of Reagents

BrdU Labeling Solution, 100 µM

Dilute the BrdU Labeling Reagent 1: 100 with sterile culture medium.

Example: For one 96-well microplate with cells cultured in 200 µL of culture medium per well, mix 20 µL of BrdU Labeling Reagent with 1.98 mL of sterile culture medium.

The 100 µM BrdU Labeling Solution is stable for 2 weeks at 4°C. For long-term storage, aliquot and store at -20°C. Protect from light.

Anti-BrdU-Eu working solution, 0.5 µg/mL

NOTE: The anti-BrdU-Eu antibody is supplied as a ready-to-use stock solution at 100 µg/mL.

Dilute the anti-BrdU-Eu stock solution 1: 200 with Assay Buffer.

Example: For one 96-well microplate, mix 60 µL of anti-BrdU-Eu stock solution with 11.94 mL of Assay Buffer.

Prepare only the amount needed within 4 hours. We advise the use of a disposable plastic container to prepare the anti-BrdU-Eu working solution.

Wash solution

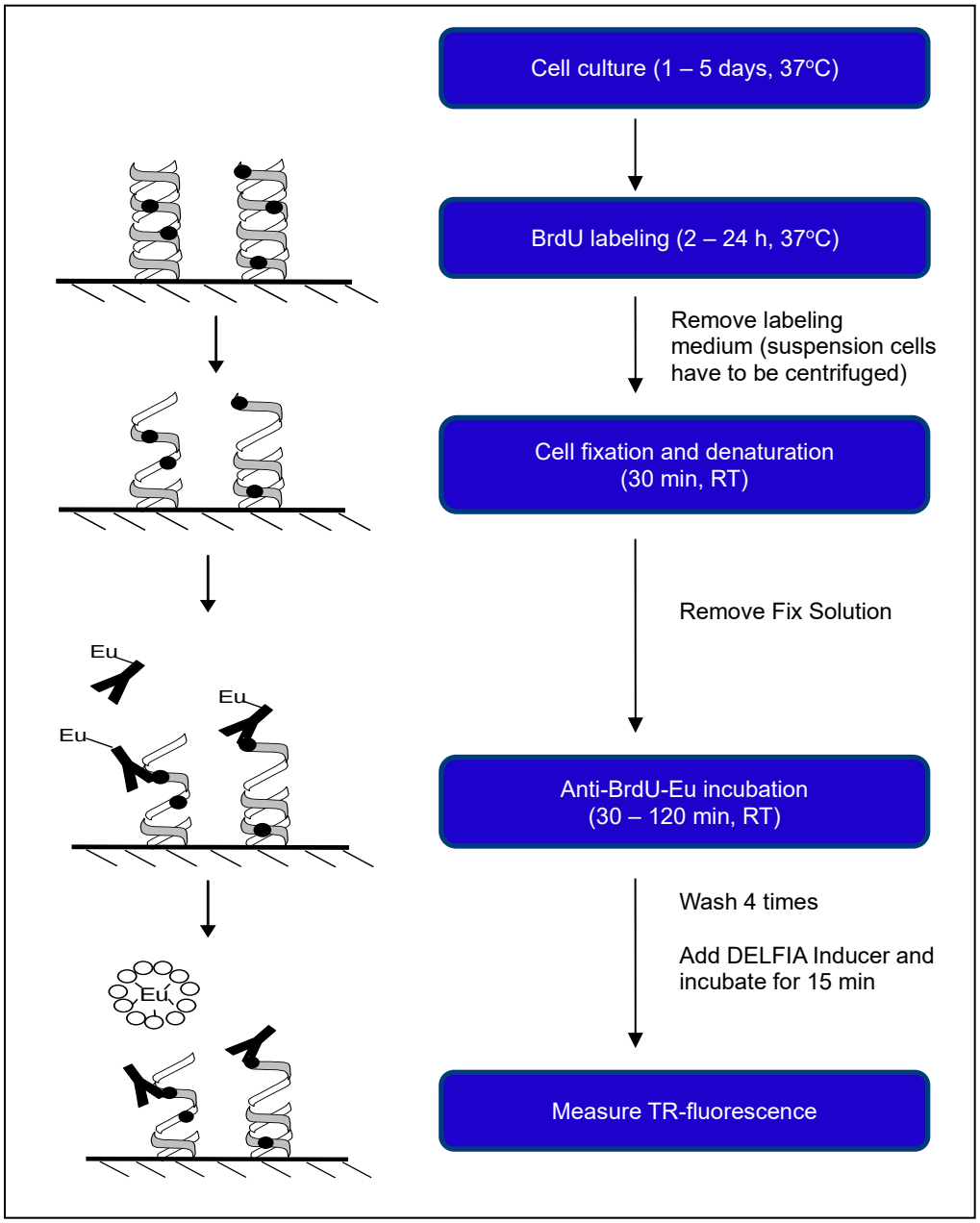
Dilute Wash Concentrate 25-fold with distilled water.

Example: For one 96-well microplate, pour 20 mL of Wash Concentrate into a clean container and add 480 mL of distilled water.

The wash solution is stable for 2 weeks at 2 - 25°C in a sealed container.

Recommended Assay Conditions

The following assay procedure is appropriate for most applications:



Procedural Notes:

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

- 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 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 added to the wells, only cells in culture medium + anti-BrdU-Eu) provide information about the unspecific binding of anti-BrdU-Eu.

Manual:

1. Plate cells in a 96-well microplate 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 hours is appropriate.

2. Label cells with BrdU by adding 1/10 volume of the 100 µM BrdU Labeling Solution to each well. For example, add 20 µL to the wells when cells are cultured in 200 µL of culture medium (except background wells) and incubate the cells for the desired time (2 - 24 h) 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. The final concentration of BrdU in the wells should be 10 µM.

3. Suspension cells have to be centrifuged at 300 $\times g$ for 10 minutes before removing the labeling medium.

4. Thoroughly remove the labeling medium without disturbing the cells

   NOTE: It is suggested to use a multichannel pipette. Carefully position the pipette tips in the corner of the well and aspirate slowly.

5. Add 100 µL of Fix Solution to each well and incubate for 30 minutes at room temperature on an orbital shaker (~100 – 120 rpm).

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

7. Add 100 µL of the 0.5 µg/mL Anti-BrdU-Eu working solution to each well and incubate for 60 minutes at room temperature on an orbital shaker (~100 – 120 rpm). Incubation time can be varied from 30 to 120 minutes.

8. Remove the Anti-BrdU-Eu working solution by inverting the plate, or using the DELFIA Platewash.

9. Wash wells 4 times with approximately 300 µL of Wash Solution per well, either manually using a multichannel pipette or using the DELFIA Platewash.

   NOTE: When washing the plates, ensure that each well is completely filled. After washing, the wells should be as dry as possible. Invert the plate and shake it to remove remaining liquid if necessary.

10. Add 200 µL of DELFIA Inducer to each well using a multichannel pipette. Flush the tips once with DELFIA Inducer and discard. Refill the tips and discard the first aliquot. Avoid touching the edge of the wells or its contents. See “Additional Note 3” on page 5 for minimizing risks of europium contamination.

    Alternatively, use the DELFIA Plate Dispense or any other automated dispensing system for the addition of the DELFIA Inducer to the wells. Make sure the tubing is flushed with DELFIA Inducer before dispensing into the wells.

11. Incubate at room temperature for a minimum of 15 minutes on an orbital shaker (~100 – 120 rpm). The fluorescence signal is stable for several hours if evaporation is prevented. However, we recommend measurement within 1 hour as on rare occasions a decrease in signal with time may be observed.

12. Measure the europium fluorescence emission in a time-resolved fluorometer.

Additional 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. For detailed information on the cleaning and maintenance of the DELFIA Platewash device, please refer to the instrument 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 a multichannel pipette (or the DELFIA Plate Dispense) after the tips have first been flushed with DELFIA Inducer. The same tips must not be used for pipetting any other reagent. When using the DELFIA Plate Dispense, please refer to the instrument’s manual.

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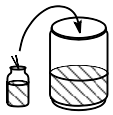
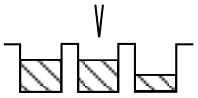
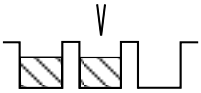
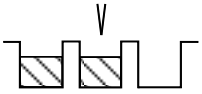
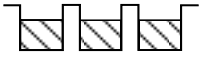
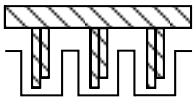
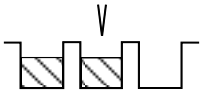
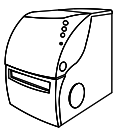
DELFIA® Cell Proliferation kit

Preparation of reagents

Prepare BrdU Labeling Solution		20 µL BrdU Labeling Reagent + 1.98 mL sterile culture medium per plate (when cells are cultured in 200 µL)
Prepare Anti-BrdU-Eu working solution		60 µL Anti-BrdU-Eu stock solution + 11.94 mL Assay Buffer per plate
Prepare wash solution		Dilute 1 : 25 with distilled water

DELFIA® Cell Proliferation kit

Summary Sheet for Assay Procedure

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 to 24 h at +37°C
Remove labeling medium		**Adherent cells: aspirate or invert and shake the plate.** Suspension cells: centrifuge at 300 × *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		60 min (30–120 min) at RT
Wash		x 4
Add DELFIA Inducer		200 µL, 15 min slow shaking
Measure fluorescence		Europium filters

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