APPLICATION NOTE

DELFIA Technology

**Key features:**

- Non-radioactive so no need for scintillation cocktail or radioactive waste disposal
- Sensitive as can detect as few as 100 cells per well
- Compatible with adherent and suspension cells
- Automatable for processing large number of samples simultaneously
- Robust with signal measurable for 8 h at room temperature

# A Cell-based Proliferation Assay for Measurement of DNA Synthesis in Microplate Format using DELFIA Technology

Identification of a compound's ADME/Tox (absorption, distribution, metabolism, elimination and toxicity) profile prior to committing it to clinical trials is essential when assessing hit compounds. Cell proliferation is an important early ADME parameter when studying live cell function, particularly in cancer and drug discovery research. Proliferation assays can be used both to quantify cell proliferation in response to growth factors, cytokines, mitogens or nutrients and to analyze cytotoxic compounds such as anticancer drugs. 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 5-bromo-2'-deoxyuridine (BrdU), a pyrimidine analog, which can be incorporated into newly synthesized DNA instead of thymidine. BrdU is detected using the DELFIA® technology.

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## 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 microliter plates. Incorporated BrdU is detected using a europium labelled 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 labelled antibody into solution, where they form highly fluorescent chelates with components of the DELFIA Inducer (Figure 1). The fluorescence measured is proportional to the DNA synthesis in the cell population of each well. The assay can be used for the direct assessment of cell numbers, and also assay for cytotoxic effects as an endpoint measurement. The assay can be used with adherent cells as well as with cells in suspension.

*Table 1.* Cells used in the optimization of the assay.

| Adherent Cells | Suspension Cells |
|---|---|
| CHO-Kt | Human lymphocytes |
| HeLa | P815 |
| HEK 293 | Jurkat |
| | CHO-S |

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:

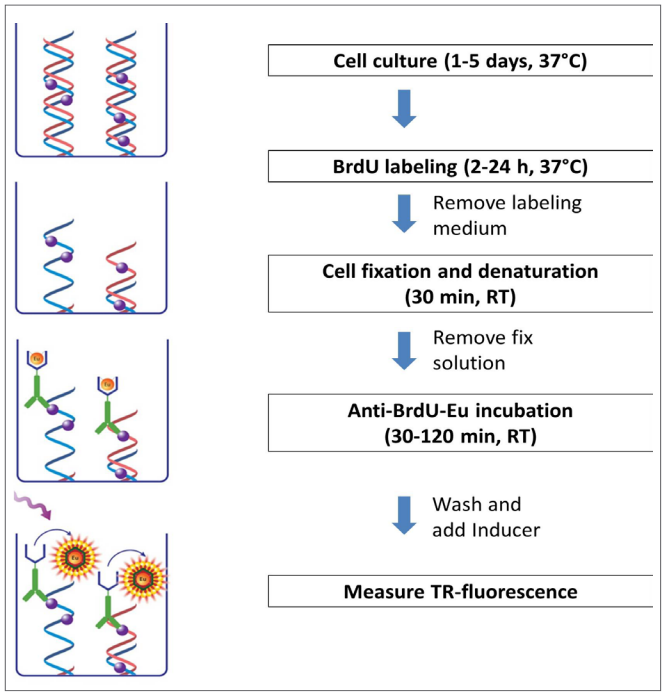


*Figure 1.* Assay procedure.

## Assay Procedure

1. Place appropriate amount of cells in a 96-well plate (at a final volume of 100 or 200 µL per well) and incubate them in the presence of various concentration of the test substance 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 to 120 h is appropriate. Two kinds of controls should also be performed to ensure the validity of the experimental setup. Information about the unspecific binding of BrdU and anti BrdU-Eu is provided by blank (no cells are added to the well, only culture medium). Information about the background of the assay is provided when no BrdU is added to the wells.
2. Label cells with BrdU by adding 10 µL/ well (if the cells were cultured in 100 µL) or 20 µL/well (if the cells were cultured in 200 µL) of BrdU Labeling Solution diluted in culture medium and reincubate the cells for additional 2 to 24 h at 37 °C in a hurnified 5% $C0_2$ atmosphere.
3. Remove labeling medium. Suspension cells have to be centrifuged at 300 x g for 10 min before removing the labeling medium.
4. Add 100 µL/well Fix Solution and incubate for 30 min at room temperature.
5 Remove Fix Solution thoroughly and add 100 µL/well Anti-BrdU-Eu working solution (0.5 11g/mL) and incubate for 30 to 120 min at room temperature.
6. Wash four times using the DELFIA Platewash.
7. Add 200 µL DELFIA Inducer directly from the reagent bottle to each well using the DELFIA Plate Dispense or Eppendorf Mullipette and shake the plate on the DELFIA Plateshake at room temperature for 15 min.
8. Measure the Eu-fluorescence in a time-resolved fluorometer.

## Results

### Titration of the number of cells

The DELFIA Cell Proliferation assay is very sensitive, less than 100 cells per well can be detected, when incubating for just 2 h with BrdU. The dynamic range for the assay is at least three orders of magnitude.

The benefits of speed, convenience and sensitivity can also be applied to cytotoxicity assays where cells can be treated with for example cytotoxic agents or cytostatic drugs.

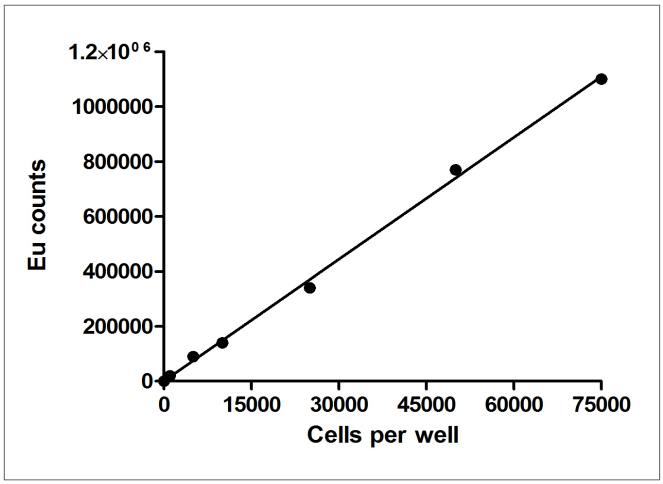


*Figure 2.* Suspension cells, P815 (mouse mast cells), were used far titrating the number of cells that con be detected by the assay. The cells were titrated in the microtiterplate at the concentrations indicated in the figure After a 24 h incubation with BrdU, the incorporation was detected as described in the Assay Procedure. Note that the cells were not grown in the microtiterplate prior to the assay, so these ore the actual numbers of cells per well.

### Measurement of the proliferation of mitogen-activated, human peripheral blood lymphocytes.

A typical application is the measurement of proliferation in mitogen-activated primary lymphocytes. The DELFIA Cell Proliferation kit can be used for measurement of proliferation in primary cells. A hundred thousand cells per well were used for an assay where the cells were grown for 48 h together with the stimulant prior to labeling with BrdU (Figure 3).

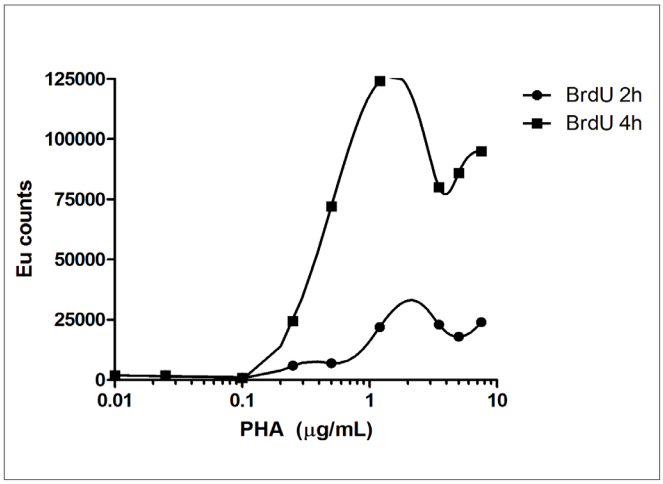


*Figure 3.* Human peripheral blood lymphocytes were isolated by density gradient centrifugation and cultured in microliter plates for 48 h in the presence of various concentrations of the mitogen phytohaemagglutinin (PHA). Subsequently, BrdU was added and the cells were reincubated for an additional 2 or 24 h. BrdU incorporation was determined as described in the Assay Procedure

### Comparison of the DELFIA Cell Proliferation assay to $^3$H-incorporation and to a colorimetric measurement of metabolism

The DELFIA Cell Proliferation kit has here been compared to the incorporation of tritiated thymidine and to a colorimetric proliferation assay. The tritiated thymidine method is similar to incorporation of BrdU, both based on the incorporation of a labeled nucleotide during DNA synthesis, while the colorimetric assay measures the metabolic activity of cellular enzymes. Figure 4 compares data obtained with the DELFIA Cell Proliferation assay, with increasing numbers of cells, to that obtained using tritiated thymidine incorporation or the colorimetric assay. The DELFIA Cell Proliferation assay shows excellent correlation with tritiated thymidine and the colorimetric assay. R-values are typically greater than 0.98. Figure 5 shows a comparison between the BrdU and tritiated thymidine incorporation for measuring proliferation in human peripheral lymphocytes, stimulated with the mitogen PHA.

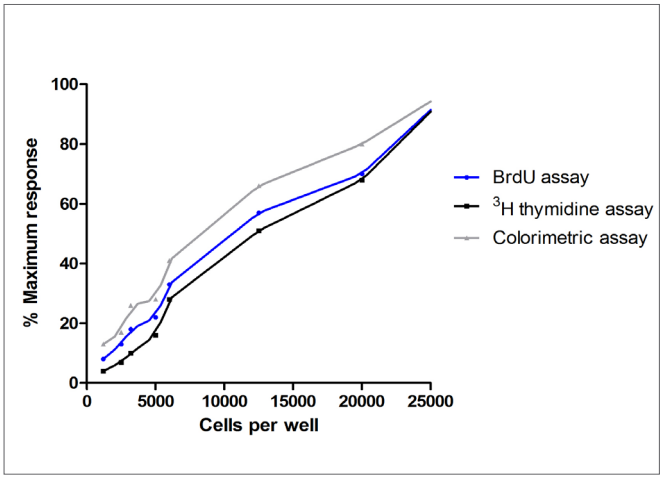


*Figure 4.* Comparison of DELFIA Cell Proliferation assay, colorimetric measurement of proliferation and tritiated thymidine incorporation with increasing number of CHO-KI cells. The cells were titrated in the microtiterplate at the concentrations indicated in the figure and grown overnight. After a 2 h incubation with BrdU or tritiated thymidine, the BrdU incorporation was detected as described in the Assay Procedure. The tritiated thymidine incorporation and the colorimetric assay were performed following standard protocols.

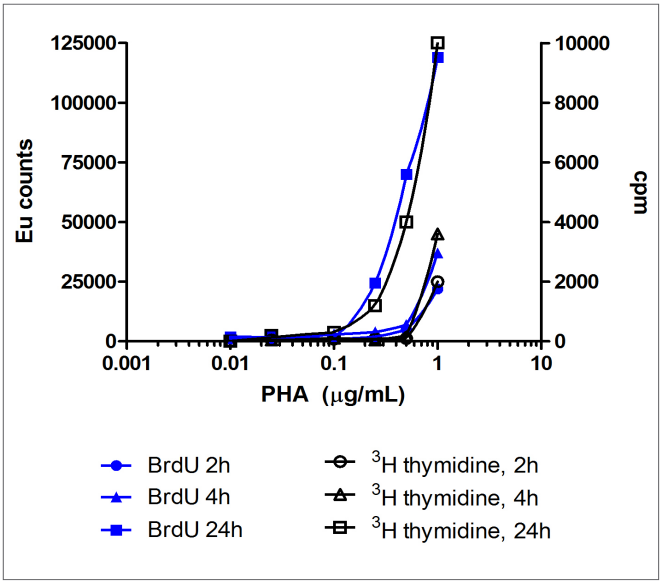


*Figure 5.* Comparison between DELFIA Cell Proliferation assay and tritiated thymidine incorporation to measure the proliferative effect of PHA in human lymphocytes. Human peripheral blood lymphocytes were isolated and cultured in microliter plates for 48 h in the presence of vatious concentrations of PHA. Subsequently, BrdU or tritiated thymidine were added and the cells were reincuboted for an additional 2, 4 or 24 h. BrdU incorporation was determined as described in the Assay Procedure. The tritiated thymidine incorporation was performed following standard protocol .

## Summary of Method Advantages

- **Non-radioactive:** Since the kit use fluorescense, it is completely safe. It overcomes the need for scintillation cocktail and radioactive waste disposal associated with $^{3}$H-Thymidine.
- **Sensitive:** Can detect as few as 100 cells per well, increased sensitivity reduces the amount of cells required per assay.
- **Works with both adherent and suspension cells:** The method works equally well with adherent and non adherent cells, whether they be cell lines or not.
- **Automation:** The assay is carried out at room temperature in a single microliter plate, no transfer of cell is needed, and a large number of samples can be processed simultaneously.
- **Robust:** The signal is measurable for 8 h at room temperature. A dry plate can be remeasured by adding 200 µL of DELFIA inducer to the well.

Products used:

* 1450-517, IsoPlates TC White 96-well Microplates
* AD0200, DELFIA Cell Proliferation kit, 960 assays
* 1296-026, DELFIA Platewash
* 1296-003, DELFIA Plateshake
* EnVision® Multilabel Plate Reader

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