Technical Note

DELFIA™ Cell Proliferation kit:

Optimized protocol for fixation of suspension cells

INTRODUCTION

Assays with suspension cells including wash steps, are challenging when it comes to fixation of the suspension cells in the wells of the micro titer plate. The DELFIA™ Cell Proliferation assay was originally optimized to be used with adherent cells. However, it can be adjusted to be used with suspension cells. The assay protocol was modified at the fixation step, so that the cells were dried in the wells prior to addition of the Fixation Solution.

In this technical note we show a new method of fixation for suspension cells. We used Jurkat cells (human T lymphocytes) throughout the study. Different cell densities and BrdU-labeling times were studied.

The DELFIA™ Cell Proliferation assay is based on the incorporation of BrdU into newly synthesized DNA strands of proliferating cells cultured in micro plates. Incorporated BrdU was detected using europium labeled monoclonal antibody. To enable antibody detection cells were fixed and DNA was denatured using Fixation Solution before addition of antibody. Unbound antibody was washed away and DELFIA Inducer was added to dissociate europium from the labeled antibody into solution, where a highly fluorescent complex is formed with the components of the DELFIA Inducer. The fluorescence measured is proportional to the DNA synthesis in the cell population of each well.

MATERIALS AND METHODS

In this study, we used the DELFIA Cell Proliferation kit (cat no AD0200) and Jurkat cells (ATCC, no TIB-152). The plates used throughout the study were white, clear bottom, 96 well plates (Costar). The cells were cultured in RPMI 1640 medium supplemented with 10% FCS (Invitrogen).

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precisely.

Protocol for suspension cells

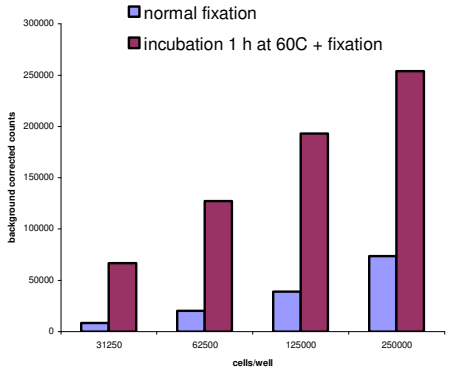
1. Place appropriate amount of cells in a 96-well plate (at a final volume of e.g. 200 µL per well) and incubate them with the substance to be tested at +37°C in a humidified 5% CO_2 atmosphere. The incubation period depends on the cell type used. For most experimental approaches, an incubation period 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. Centrifuge the plate at 300 x g for 10 minutes before removing the labeling medium. **After removing the medium, incubate at 60°C for 1 hour, or until the wells are dry.**
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.
8. Add 200 µL DELFIA Inducer to each well using the DELFIA Plate Dispense or Eppendorf Multipipette. When using the DELFIA Plate Dispense make sure that the tubing is flushed with DELFIA Inducer before dispensing into the well. 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.
9. Measure the Eu-fluorescence in a time-resolved fluorometer, like VICTOR^{2}V™ or the EnVision™ using parameters recommended in the kit manual.

RESULTS

Effect of drying the cells in the wells prior to fixation

The effect of drying the BrdU-labeled suspension cells for 1 hour at 60°C before adding the Fixation Solution was studied.

A)



B)

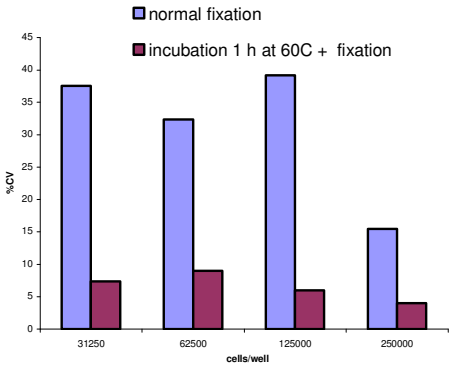
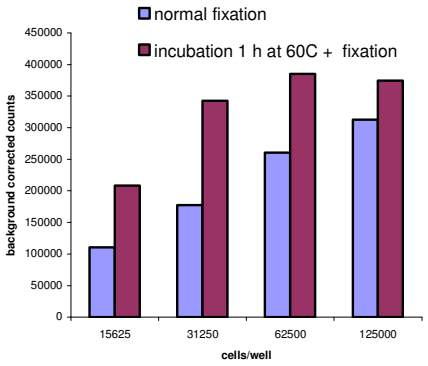


***Figure 1**. Jurkat cells were plated at different densities and labeled with BrdU for 2 hours. A comparison was made where one plate was incubated for 1 hour at 60°C after removal of the BrdU-labeling medium and the other was not. The data is presented A) as counts (corrected for the background values from cells without BrdU), and as B) % CV (n=6).*

Optimization of cell density for longer BrdU-labeling

Studies of the effect that certain compounds have on the cells over longer periods of time may require labeling of the cells with BrdU overnight, or longer. The cell density can be optimized to improve the fixation of suspension cells during long BrdU-labeling incubation.

A)



B)

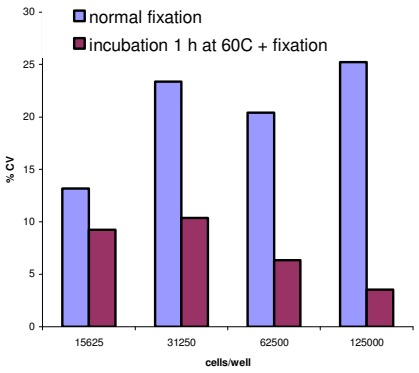


Figure 2*. Jurkat cells were plated at different cell densities and labeled with BrdU for 24 hours. A comparison was made where one plate was incubated for 1 hour at 60°C after removal of the BrdU-labeling medium ant the other was not. The data is presented both as A) counts (corrected for the background values from cells without BrdU), and as B) % CV (n=6).*

In conclusion, we found that incubating the cell plate for 1 hour at 60°C, or until the wells are completely dry, before addition of the Fixation Solution improved the assay performance. Using Jurkat suspension cells, the drying step improved the assay CV from 15 – 40% to 4 – 10%. The drying of the cells also improved the signal levels considerably. Choosing a suitable cell density for long-term incubations with BrdU also improves the assay performance when using suspension cells.