Introduction

DELFIA® time-resolved fluorescence (TRF) assays are widely used in research and drug discovery, not only because they are robust, but also because they have other advantages over conventional ELISA. The unique chemical properties of lanthanide chelates in combination with time delayed signal measurement result in flexible assays with high sensitivity, wide dynamic range, and superior stability. Its wide variety of applications includes cell cytotoxicity, proliferation assays, kinases and receptor-ligand binding assays.

The EnSpire® Multimode Plate Reader is the only plate reader combining conventional labeled detection technologies with Corning® Epic® label-free technology. A key feature is the choice of combinations of the various reading technologies available to the user, offering a well-tailored solution suitable to every research lab or drug company. In addition, the EnSpire platform combines filter based excitation and dual-monochromator-based emission detection of TRF samples offering both high sensitivity and versatility. The ability to adjust the emission wavelength with dual monochromators is an extremely powerful option for custom-designed TRF assays. The ideal parameters can be determined in wavelength scans and set to the specific needs of a wide range of acceptor fluorophores. Moreover, it supports future developments of complex assay types with multiple fluorophores.

In this study, the suitability of the EnSpire Multimode Plate Reader for DELFIA assays is investigated using the example of a cell-based DELFIA proliferation assay. Proliferation is an important parameter when studying live cell function, particularly in cancer and drug discovery research, e.g. to study growth factors, cytokines or mitogens, and also proliferation inhibitors such as those found in anti-cancer drugs. Here, we show the high sensitivity of this assay in a cell titration experiment as well as proliferation inhibition via reductions of serum concentration in the cell media.
**Materials and Methods**

**DELFIA 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 microtiter plates. Incorporated BrdU is detected using a 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 (Figure 1). The fluorescence measured is proportional to the DNA synthesis in the cell population of each well. The assay can be used with adherent cells as well as with cells in suspension.

**Cell and sample preparation**

All experiments were performed with adherent cells, HeLa and CHO-K1, in white 96-well plates (DELFIA plates, PerkinElmer). Cells were cultured according to standard laboratory protocols. Cell preparation was performed for both experiments strictly following the kit manual, incubating cells for at least 2 hours with BrdU, depending on the respective experiment.

For the cell titration experiments of the assay, both cell lines were titrated into the microplate. HeLa cells were seeded at a density of 1.8x10^4 to 1.8x10^5 cells per well for 24 hours before incubation with BrdU, while CHO-K1 cells were incubated at a density of 5.4x10^1 to 1.7x10^6 cells per well immediately after titration.

For proliferation inhibition cells at 1.6x10^4 (HeLa) / 9.6x10^3 (CHO-K1) cells per well were incubated for 48 and 72 hours respectively. The cell medium contained an increasing concentration of fetal calf serum (FCS) of 0 to 10%, where 10% is the concentration in the standard cell medium.

**EnSpire parameter settings**

Detailed parameter settings for these experiments are shown in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Instrument settings.</th>
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<td><strong>EnSpire settings</strong></td>
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<td>Excitation filter</td>
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Prior to the DELFIA assays, the sensitivity was determined with 100nM Eu-solution (Cat#:B119-100) to be 150fM which equals 5 amol/well.

**Results and Discussion**

**Cell titration**

To test the sensitivity of the DELFIA Cell Proliferation Assay on the EnSpire, titrations of adherent cells (HeLa and CHO-K1) were performed. The results of the titration from both cell lines (Figures 2 and 3) show that as few as 100 cells per well can be detected when incubating for a mere 2 hours with BrdU. The dynamic range for the assay is at least 3 orders of magnitude.

The flattening of the titration curve of HeLa cells clearly shows the start of inhibition of proliferation of the cells in higher-density wells after the 24-hour growing period before assay preparation (Figure 2). However, for the CHO-K1 cells, which were incubated with BrdU and measured directly after titration, the linear increase rising from the titration concentration can be detected (Figure 3).
Measurement of the proliferation inhibition

Proliferation inhibition was studied by reducing the serum concentration of the cell medium. This causes a lack of growth factors and so prohibits cell division.

The adherent cells were plated 48 (HeLa) or 72 (CHO-K1) hours before incubating with BrdU at a medium cell density in cell medium containing different concentrations of FCS. Both cell lines react clearly to the lack of serum in the medium (Figures 4 and 5). The effect of inhibiting cell proliferation is more pronounced when a longer growing cycle (in this example 72 hours for CHO-K1 cells) is used. This is because there are more division cycles and a longer lack of serum. The lack of serum only shows its full effect after a certain period of time due to the cell’s internal reserves during the first hours of starvation.

Conclusion

The data obtained show that the TRF mode on EnSpire is suitable for cellular and biochemical DELFIA assays as shown using the example of a DELFIA cell proliferation assay. As few as 100 cells per well can be detected and the dynamic range is at least 3 orders of magnitude in these tests. This example assay and the sensitivity, as determined from the Eu-solution, show that the EnSpire is well suited for all common TRF applications. The use of monochromator technology provides additional flexibility in the choice of acceptor dyes for a wide range of TR-FRET reagents.