Cell Based Assays for Studying the Effects of Cytotoxic Agents

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1

Apoptosis, DNA fragmentation and cell proliferation are important parameters when studying live cell function, especially, when the effects of cytotoxic agents are under study. We have developed sensitive assays to monitor these events. The TruPoint™ Caspase-3 assay is a robust and homogenous assay that can be used with purified enzyme, apoptotic cell lysates or whole cells. The assay can be easily automated and run in different plate formats (96 – 1536), with small amounts of enzyme or cells, thus reducing costs. The DELFIA® Cell Proliferation assay measures the effects of growth regulatory substances. It is a time-resolved fluoroimmunoassay based on the incorporation of BrdU into newly synthesised DNA strands of proliferating cells grown in microtiter plates. Once incorporated into DNA, the labeled nucleotides can be detected by Europium labeled antibody. The DELFIA® DNA Fragmentation assay is a cell-based TUNEL assay performed in 96 well microplate format for quantitative detection of apoptosis.

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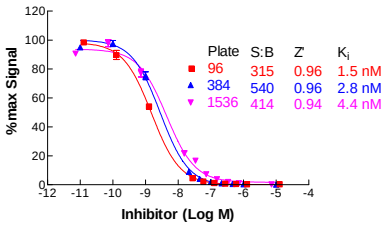
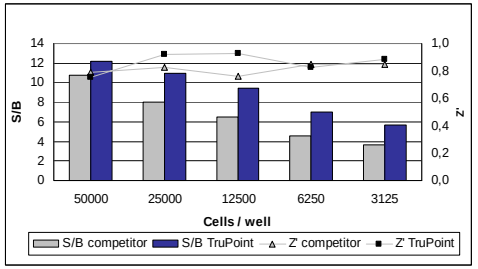
Caspase-3 assay using purified enzyme in different plate formats



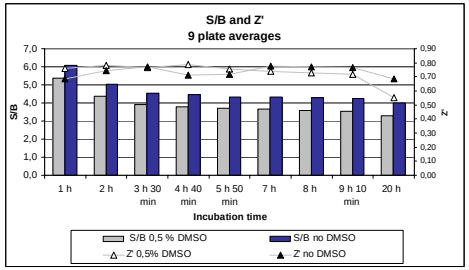
Caspase-3 inhibition assay with purified enzyme using three different assay formats

Caspase-3 assay using whole cells



Caspase-3 assay using different numbers of cells per well. S/B ratios are calculated as the ratio of signal from staurosporin treated cells to signal from untreated cells. The assay volume was 50 µl for both TruPoint and competitor assay. The signal was measured after 30 minutes using 2100 EnVision Multilabel Reader.

Automated Caspase-3 assay in 384-well format using whole cells



TruPoint Caspase-3 assay was run in 384-well format (9 plate batch) in an automated workstation using whole cells to study the effect of DMSO and.assay stability .

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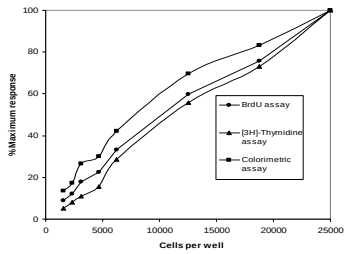
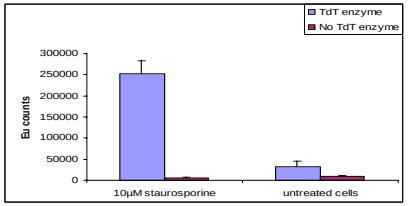
Comparison of the DELFIA Cell Proliferation assay to 3H-incorporation and to a colorimetric measurement of metabolism



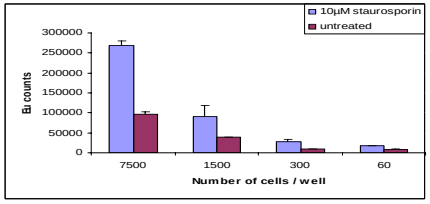
Comparison of DELFIA Cell Proliferation assay, colorimetric measurement of proliferation and tritiated thymidine incorporation with increasing number of CHO-KI cells. The cells were titrated in the microtiterplate at the concentrations indicated in the figure and grown overnight. After a 2 h incubation with BrdU or tritiated thymidine, the BrdU incorporation was detected as described in the kit assay procedure. The tritiated thymidine incorporation and the colorimetric assay were performed following standard protocols. All the assays showed linear response to cell number. R-values greater than 0.98 were obtained.

4

DNA Fragmentation assay in a cell-based TUNEL assay performed in 96 well microplate format



The results show the amount of DNA fragmentation in CHO cells treated 6h with 10µM staurosporin compared to untreated cells. The cells were fixed and the TUNEL reaction was performed at 37 °C. The incorporated Bio-dUTP was detected using Eu-labelled streptavidin. The fluorescence was measured using VICTOR™.



Detection of apoptosis in CHO cells. The amount of fragmented DNA is dependent on the number of cells. The cells were fixed and the TUNEL reaction was performed at 37 °C. The incorporated Bio-dUTP was detected using Eu-labelled streptavidin. The fluorescence was measured using VICTOR™.