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A Cell-Based DNA Fragmentation Assay in Microplate Format using DELFIA® Technology

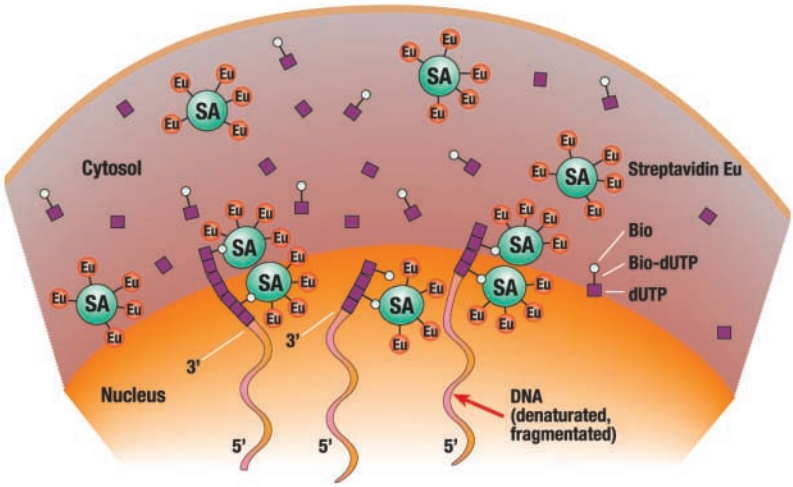
Sofia Vikström, Heini Frang, Jarkko Karvinen, Pertti Hurskainen and Ilkka Hemmilä

PerkinElmer Life Sciences, Wallac OY, FIN-20101 Turku, Finland

1 Introduction

DNA fragmentation has long been used to distinguish apoptosis from necrosis, and is among the most reliable methods for detection of apoptotic cells. When DNA strands are cleaved or nicked by nucleases, 3'-hydroxyl ends are exposed. The hydroxyl ends can then serve as a starting point for terminal deoxynucleotidyl transferase (TdT), which adds deoxyribonucleotides in a template-independent fashion. Addition of labelled dUTP to the TdT reaction thus provides a means to label the DNA strand breaks. Once incorporated into DNA, the labelled nucleotide can be detected by Europium labeled streptavidin. This method of labeling DNA breaks is referred to as terminal deoxynucleotide transferase dUTP nick end labeling, or TUNEL[1].

The aim of the present study was to set up a DELFIA® time-resolved fluorescence assay to test substances for their apoptotic effect on cells. The DELFIA DNA Fragmentation assay is performed in 96-well microplate format for quantitative detection of apoptosis.



2 Methods

CHO cells, grown in RPMI medium supplemented with 10 % FCS and 1 % Penicillin & Streptomycin (Life Technologies), were seeded in 96-well plates and grown overnight. The cells were treated with 10 µM staurosporine (Sigma) for 6 h at 37ºC (CO_2-incubator). After treatment, the growth medium was removed and 100 µL/well Fixation Solution was added. The cells were fixed 30 minutes at room temperature. The Fixation Solution was tapped off and the following reaction was started: 0.01 % CHAPS, 5.5 U TdT enzyme (Amersham), 15 µM dTTP, 5 µM Bio-dUTP (Roche) and TdT buffer (Amersham, 500 mM sodium cacodylate pH 7.2, 10 mM $CoCl_2$, 1 mM 2-mercapthoethanol), in a final reaction volume of 50 µL. A control reaction without the enzyme was also started. The reaction was performed at 37ºC for 30 minutes. The plate was washed 6 times using the DELFIA Platewash. Europium (Eu)-labelled streptavidin was added to the wells at a concentration of 100 ng/mL in 50 µL. The Eu-labelled streptavidin was diluted in DELFIA Assay Buffer. The plate was incubated 1 hour at room temperature and was washed 6 times prior to addition of 200 µL/well Enhancment Solution. The plate was shaked for 5 minutes and the fluorescence was measured using a VICTOR™ Multilabel Counter.

3 Results

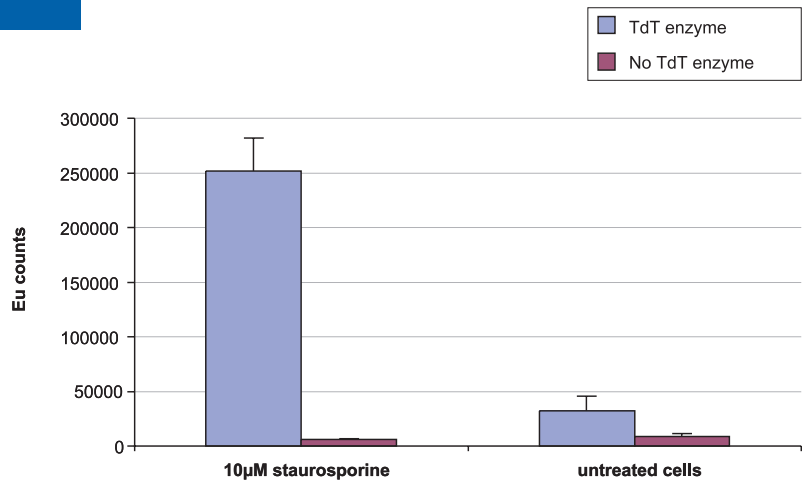


Figure 1. The results show the amount of DNA fragmentation in CHO cells treated 6h with 10 µM staurosporin compared to untreated cells. The cells were fixed and the TUNEL reaction was performed at 37ºC. The incorporated Bio-dUTP was detected using Eu-labelled streptavidin. The fluorescence was measured using a VICTOR Multilabel Counter.

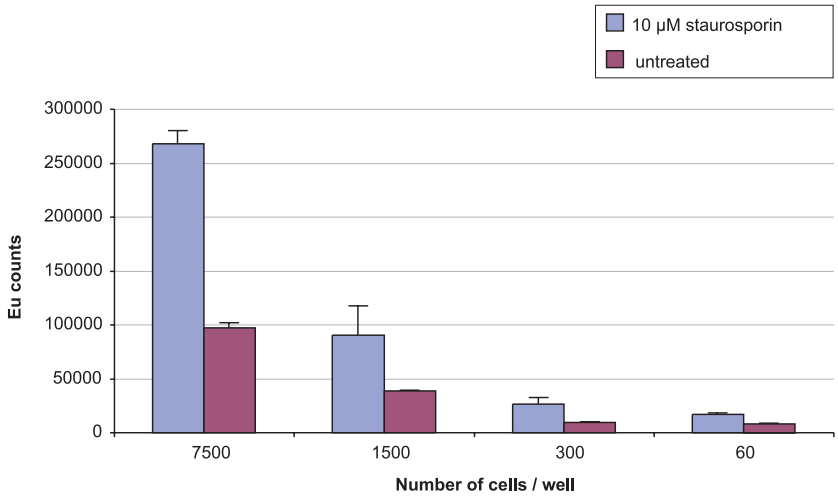


Figure 2. Detection of apoptosis in CHO cells. The amount of fragmented DNA is dependent on the number of cells. The cells were fixed and the TUNEL reaction was performed at 37ºC. The incorporated Bio-dUTP was detected using Eu-labelled streptavidin. The fluorescence was measured using a VICTOR Multilabel Counter.

4 Conclusions

The data demonstrates that the DELFIA DNA Fragmentation assay is a simple and fast microplate format method, for detection of DNA strand breaks in cells. This is a sensitive and efficient method for screening potential inducers of apoptosis.

Products used:

- Wallac Isoplates TC 1450-517
- DELFIA Assay Buffer 1244-111
- Wallac Eu-SA 1244-360
- DELFIA Wash Concentrate 1244-114
- DELFIA Platewash 1296-026
- DELFIA Enhancement Solution 1244-105
- DELFIA Plateshake 1296-001/002 or 1296-003/004
- Wallac 1420 VICTOR^2 Multilabel Counter

5 Reference

Gavrieli, Y., Sherman, Y. and Ben-Sasson S.A. (1992). Identification of programmed cell death in situ via specific labelling of nuclear DNA fragmentation. *J. Cell. Biol.* **119**, 493–501

Worldwide Headquarters: PerkinElmer Life Sciences, Inc., 549 Albany Street, Boston, MA 02118-2512 USA (800) 551-2121

European Headquarters: PerkinElmer Life Sciences, Inc., Imperiastraat 8, BE-1930 Zaventem Belgium

Technical Support: in Europe: techsupport.europe@perkinelmer.com in US and Rest of World: techsupport@perkinelmer.com

Belgium: Tel: 0800 94 540 • **France:** Tel: 0800 90 77 62 • **Netherlands:** Tel: 0800 02 23 042 • **Germany:** Tel: 0800 1 81 00 32 • **United Kingdom:** Tel: 0800 89 60 46

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