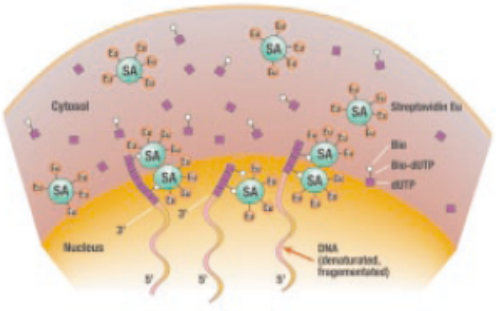
Application Note

A new cell-based DELFIA® DNA Fragmentation assay for testing apoptotic effect of lead compounds

INTRODUCTION

The DELFIA® DNA Fragmentation assay is performed in 96-well microplate format for quantitative detection of apoptosis. It is a simple and upscalable method to test substances for their apoptotic effect on cells.

During the process of apoptosis, DNA fragmentation occurs following the activation of endonucleases. The labeling of the 3' ends of DNA fragments provides an easy measure of cells undergoing apoptosis. Modified nucleotides are incorporated at the 3' ends by the activity of terminal deoxynucleotidyl transferase (TdT).



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. These 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].

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 37°C (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, 500mM 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 37°C for 30 minutes. The plate was washed 6 times using the DELFIA Platewash. 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 shaken for 5 minutes and the fluorescence was measured using the VICTOR²™ plate reader.

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RESULTS

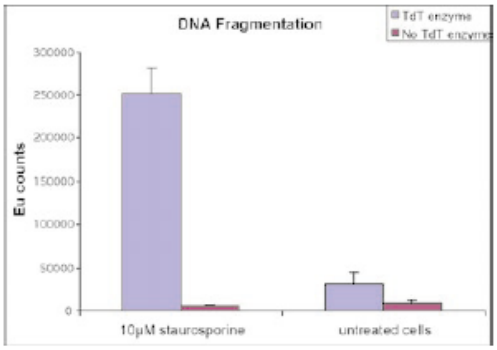

Fig.1. The results show the amount of DNA fragmentation in CHO cells treated 6h with 10µM staurosporin compared to untreated cells. The cells were fixed and the TUNEL reaction was performed at 37°C. The incorporated Bio-dUTP was detected using Eu-labelled streptavidin. The fluorescence was measured using the VICTOR²™ fluorescence was measured using the VICTOR²™ plate reader.

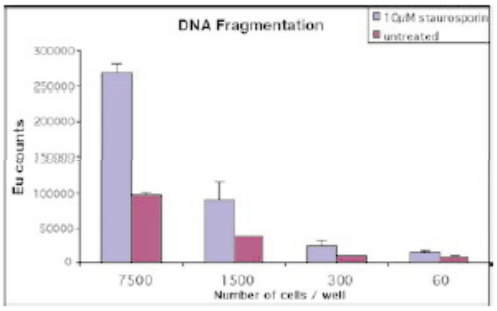

Fig.2. Detection of apoptosis in CHO cells. The amount of fragmented DNA is dependent on the number of cells. The cells were fixed and the TUNEL reaction was performed at 37°C. The incorporated Bio-dUTP was detected using Eu-labelled streptavidin. The fluorescence was measured using the VICTOR²™ plate reader.

The data demonstrate 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.

REFERENCES:

1. 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

Catalog Items: (see DELFIA buffers guide)

Cat.No.	Products	Size
1244-360	DELFIA Eu-labelled streptavidin	0.25 mg
	streptavidin	
1244-114	DELFIA Wash Concentrate	250 ml
1244-111	DELFIA Assay Buffer	250 ml
1244-105	DELFIA Enhancment Solution	250 ml
1450-516 1450-517	Wallac Isoplates TC	25/100 plts
1296-026	DELFIA Platewash	
1296-001/002 1296-003/004	DELFIA Plateshake	
Wallac 1420	VICTOR² Plate Reader	

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