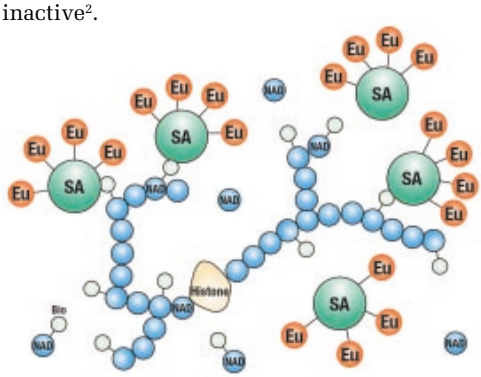
Application Note

A DELFIA® Assay for Inhibition of PARP

The DELFIA time-resolved fluorescence assay for inhibition of poly(ADP-ribose) polymerase (PARP) is a 96-well microplate assay that measures the inhibition of PARP-activated incorporation of biotinylated nicotinamide adenine diphsophate (NAD). The incorporation is detected using Europium-labelled streptavidin. The method is a convenient way to screen test compounds for PARP activity in high throughput primary screening and secondary screening.

Poly(ADP-ribose) polymerase (PARP) is a nuclear protein involved in the response to DNA damage, where it catalyzes the polymerization of nicotinamide adenine diphsophate (NAD) into chains of poly(ADP-ribose) polymers[1]. The polymerization occurs on a number of nuclear proteins, as well as on the PARP protein itself. Following activation by DNA strand breaks, PARP hydrolyzes NAD and catalyzes the formation of PARP onto itself and other nuclear proteins, with the release of nicotinamide. PARP binds to single- and double-stranded DNA breaks. When the enzyme undergoes auto-poly (ADP-ribosyl)ation, the automodified PARP dissociates from the DNA and becomes inactive[2].



Inhibitors of PARP have been shown to help prevent ischemic injury in the brain, increase apoptosis in cancer cells, prevent infiltration of neutrofils and subsequent inflammation, and to suppress the production of nitric oxide synthase in macrophages[3,4]. In response to low to moderate levels of DNA damage, PARP is activated and NAD levels in the cells decrease. Experiments with PARP inhibitors suggest that PARP activity may be necessary to rescue cells after low to moderate levels of DNA damage. In contrast, when cells experience massive levels of DNA damage and DNA strand breaks, activation of PARP can lead to depletion of NAD and ATP, resulting in a marked decrease in energy-dependent processes and in DNA repair. In this situation, activation of PARP by massive DNA damage processes may actually be a suicide response, since it causes rapid NAD and ATP depletion and leads to cell death before the cell can repair the DNA damage.

METHODS

The DNA used in the polymerization reaction was fragmented by hydrodynamic shearing. The DNA was passed through the orifice of a 28-gauge hypodermic needle. This method of shearing generates 1.5-2.0 kb DNA fragments[5]. The polymerization reaction was performed in an uncoated 96-well microtiter plate. To the well was added: 2.5 µg histones (Trevigen/Upstate U.C. Biotechnology), 1 µg DNA (Sigma), 15 µM NAD + Bio-NAD (Trevigen) in a 17:1 or 1:1 ratio, 1 mM 3-aminobenzamide[6] (inhibitor of PARP, Sigma) 0.5 U PARP enzyme (Trevigen) and PARP

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buffer (500 mM Tris-HCl, 250 mM $MgCl_2$). The reaction was performed at room temperature, for 30 minutes. The plate was then washed 2 times using the DELFIA Platewash. Eu-labelled streptavidin was diluted in DELFIA Assay Buffer to 200 ng/well and incubated for 30 minutes at room temperature. The plate was washed 4 times using the DELFIA Platewash, prior to addition of 200 µL/well DELFIA Enhancment Solution. The fluorescence was measured using VICTOR$^2_{TM}$ plate reader.

RESULTS

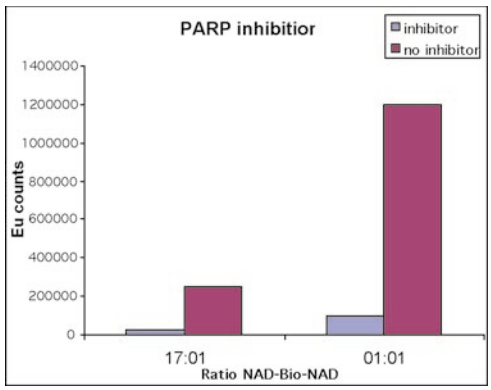

Fig.1 PARP inhibition. The inhibitor 3-amino-benzamide was used for inhibition of PARP activity. The reaction was performed in 96-well microplate format. 15 µM NAD/Bio-NAD and 0.5 U PARP enzyme reacted in the presense of 2.5 µg histones and 1 µg sheared DNA. The incorporation of biotin labelled NAD was detected using Eu-labelled streptavidin and the fluorescence was measured.

The data shows that the DELFIA time-resolved fluorescence assay for inhibition of PARP allows for the measurement of the incorporation of biotinylated NAD. This technology is therefore suitable for the screening of inhibitors of PARP where the formation of poly(ADP-ribose) chains is inhibited.

REFERENCES:

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Catalog Items: (see DELFIA buffers guide)

Cat.No.	Products	Size
1244-360	DELFIA Eu-labelled streptavidin	0.25 mg
1244-111	DELFIA Assay Buffer	250 ml
1244-114	DELFIA Wash Concentrate	250 ml
1244-105	DELFIA Enhancement Solution	250 ml
AAAND-0001	DELFIA yellow plates, 96 wells	60 plts
1296-026	DELFIA Platewash	
1296-001/002 1296-003/004	DELFIA Plateshake	
Wallac 1420	VICTOR² Plate reader	

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1234-9865-01, Aug 2002 Printed in Finland by Offset House Oy Naantali 2002