PerkinElmer®
precisely.

A Cell-based DELFIA Assay for Measuring Phosphorylation of Proteins

Sofia Vikström, Christel Gripenberg-Lerche, Pertti Hurskainen and Ilkka Hemmilä

PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland

1 Introduction

We have set up a cell-based DELFIA® assay in 96-well microplate format, to test compounds or conditions for their ability to modulate cellular stress. The phosphorylation of MAP kinase is used as a tool for screening compounds and conditions that induce, or reduce cellular stress. The assay replaces classical gel-electrophoresis and Western blotting techniques by the utilization of cells as the target of the assay instead of isolated proteins. The assay detects changes in the phosphorylation level using a europium-labeled antibody. Additionally, the binding to another cellular component is used as a control of equal number of cells in the well. For this assay we used samarium-labeled wheat germ agglutinin (WGA).

2 Methods

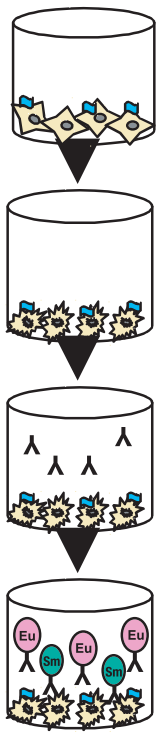
CHO-K1 cells were grown in RPMI culture medium supplemented with 10% serum (Invitrogen). Prior to the assay, the cells were plated in 96-well plates, Isoplate TC, or ViewPlate, and grown o/n.

The culture media was removed, and the cells were treated with 0.6% H_2O_2 in PBS 20 mM HEPES for different periods of time at 37°C. Control cells were in PBS 20 mM HEPES. The cells were washed once with PBS 20 mM HEPES after the treatment. Immediately after the treatment, the cells were fixed and permeabilized by addition of 100 µL/well 4% Formaldehyde, 0.1% Triton in PBS, for 30 min at RT, then washed once with PBS.

A monoclonal anti-phospho-p44/42MAPK antibody (Cell Signaling # 9106B) was diluted to 300 ng/mL in DELFIA Assay Buffer supplemented with 0.5% BSA giving totally 1% BSA. The cells were incubated with 100 µL/well for 1 - 2 hours at RT. The plate was washed two times with the DELFIA Plate Wash using DELFIA Wash solution.

DELFIA Eu-anti mouse antibody was diluted to 300 ng/mL, and Sm-WGA (WGA from Vector Laboratories, labeled with samarium at Wallac Labeling Service) was diluted to 1 µg/mL in DELFIA Assay Buffer containing 1% BSA and 2 mM $CaCl_2$. The plate was incubated with 100 µL/well for 1 - 2 hours at RT.

The plate was washed four times on the DELFIA Plate Wash using DELFIA Wash solution. 200 µL/well DELFIA Enhancement Solution was added and the plate was shaken for 5 minutes on the DELFIA Plateshake. The plate was then measured using Wallac 1420 VICTOR^2 Plate reader, with the Europium & samarium dual label protocol.



Grow cells in 96-well tissue culture plate.
Activate kinase, and/or add test substances.

Remove culture media, fix and permeabilize the cells in the wells.

Add primary antibody, incubate and wash.

Add Eu-labeled antibody, and Sm-labeled WGA, incubate, wash, and measure the time-resolved fluorescence.

3 Results

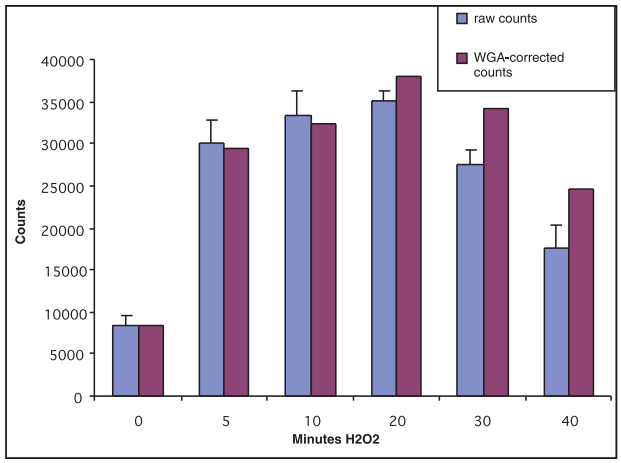


Figure 1. H_2O_2 which is an inducer of oxidative stress, induces a time-dependent phosphorylation of MAP kinase in CHO cells grown in a 96-well plate. The graph shows the raw counts, compared to counts that have been corrected for the number of cells per well, using Sm-labeled WGA.

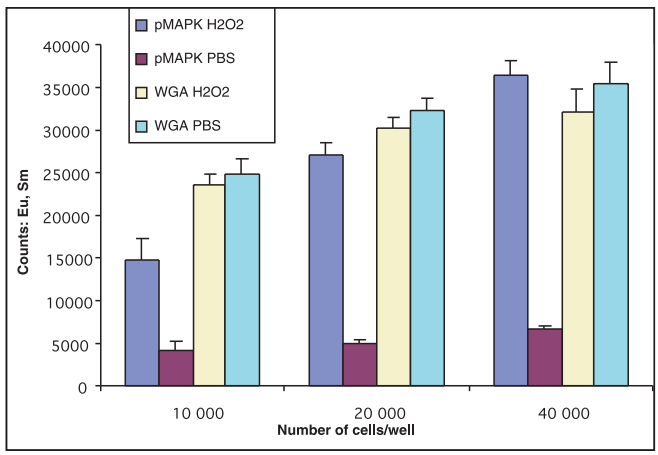


Figure 2. Detection of phosphorylated MAP kinase in CHO cells grown on a 96-well plate. H_2O_2 which is an inducer of oxidative stress, induces phosphorylation of MAP kinase. The phosphorylated form of MAPK (pMAPK) was detected using a monoclonal anti-phospho-p44/42MAPK antibody and Eu-labeled anti-mouse antibody. The number of cells per well correlates to the amount of phosphorylation and the reduction of cells per well due to H_2O_2 treatment can be corrected for by the Sm-labeled WGA counts.

4 Conclusions

We conclude that the cell-based DELFIA assay is a reliable method for detecting the phosphorylation, and thus the activation, of MAP kinase, in cells grown on 96-well plates. Because MAP kinase is activated in response to cellular stress, the cell-based DELFIA assay is suitable for testing compounds that modulate oxidative stress.

This cell-based DELFIA assay provides a means for multi-label assays. The same approach could be used for detecting at least three different kinases or other events in the cell, when using one of the lanthanide labels as a control for the number of cells.

5 PerkinElmer products and services:

- Wallac Isoplates TC 1450-517
- View plate 6005181
- DELFIA Assay Buffer 1244-111
- DELFIA Wash Concentrate 1244-114
- DELFIA Platewash 1296-026
- DELFIA Eu-N1 labeled anti-mouse antibody AD0124
- DELFIA Enhancement Solution 1244-105
- DELFIA Plateshake 1296-001/002 or 1296-003/004
- Wallac 1420 VICTOR^2 Plate reader
- Wallac Labeling Service (labellingservices@perkinelmer.com)

PerkinElmer Life and
Analytical Sciences
710 Bridgeport Avenue
Shelton, CT 06484-4794 USA
Phone: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®
precisely.

For a complete listing of our global offices, visit www.perkinelmer.com/lasoffices

006942_01 Printed in USA