

# Application Notes

## DELFI<sup>®</sup> Protein Kinase assays

Protein kinases use ATP to phosphorylate certain amino acid residues (tyrosine, serine or threonine) on their substrates. In receptor-mediated signalling the phosphorylation forms an important secondary messenger system. Phosphorylation can be measured following radioactive <sup>32</sup>P incorporation, which, however, involves the problems of radioactive work, safety and disposal. Eu-labelled anti-phosphotyrosine, -threonine and threonine-proline are available for the sensitive detection of phosphorylation levels on various substrates using time-resolved fluorometry and DELFI<sup>®</sup> as the detection technology.

### PROTOCOL

Convenient collection of the substrates (peptides, polypeptides or proteins) on coated microtitration plates can be accomplished using streptavidin plates for biotinylated substrates or glutathione or anti-glutathione-S-transferase plates for substrates expressed with a GST tag.

1. In the assay, first incubate the substrate with enzyme (receptor), ATP and effectors.
2. After phosphorylation collect the phosphorylated products on coated plates. Incubate 1-2 hours and wash the plate two or three times with wash solution.
3. Add Eu-(or biotin-) labelled antibodies in DELFI Assay Buffer (200 µl, 250 ng/ml) and incubate for 1-2 h. If performed in the indirect way, the secondary incubation with Eu-streptavidin takes 20-30 minutes.

4. Wash the plate six times with wash solution using the DELFI Platewash (1296-024 or 026).

5. Add 200 µl of DELFI Enhancement Solution, shake for 10 min with Plateshaker (1296-001/002 or 003/004) to guarantee a stable signal level and measure the plate with the Wallac time-resolved fluorometer.

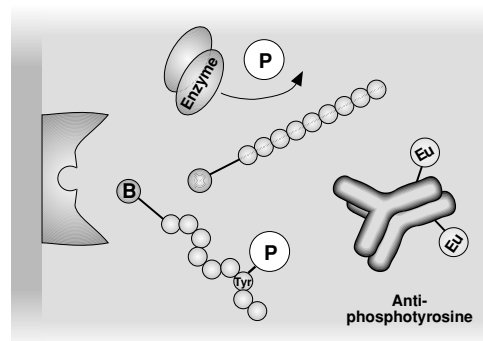


FIG.1. Detection of phosphorylation using Eu-labelled anti-phosphotyrosine and streptavidin-coated microtitration plates.

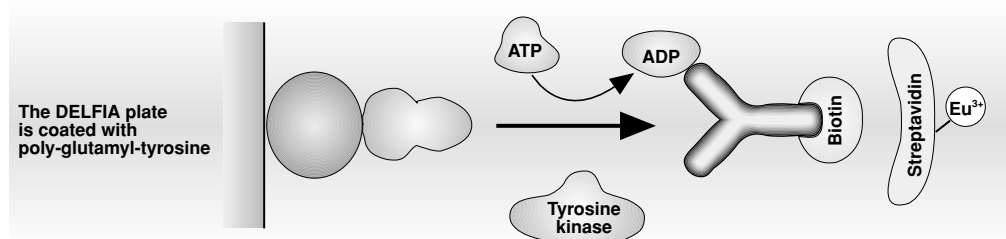


FIG.2. Schematic assay for detection of phosphorylation using Eu-labelled streptavidin for quantitation of biotinylated anti-phosphotyrosine antibody. For more information see Braunwalder et al.

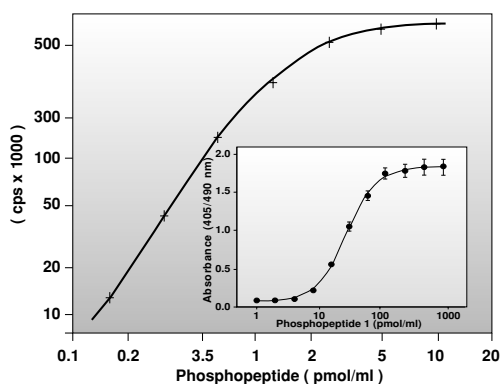


FIG.3. Quantitation of phosphorylated phosphopeptide using Eu-labelled anti-phosphotyrosine.

- |              |   |
|--------------|---|
| 1244-360     | Eu-labeled streptavidin, (for 50-125 plates)      |
| 1244-111     | DELFLA Assay Buffer, 250 ml (for 5 plates)        |
| 4002-0010    | DELFLA Assay Buffer, 1000 ml                      |
| 1244-114     | DELFLA Wash concentrate, 25 x concentrate, 250 ml |
| 1244-105     | DELFLA Enhancement Solution, 250 ml               |
| 4001-0010    | DELFLA Enhancement Solution, 1000 ml              |
| 1420         | VICTOR <sup>2</sup> , Multilabel counter          |
| 1296-026     | DELFLA Platwash                                   |
| 1296-003/004 | DELFLA Plateshake                                 |
| 1296-014     | Multipette  |
| 1296-016     | Combitips, 5 ml, pack of 100                      |
| 1296-017     | Combitips, 2.5 ml, pack of 100                    |

## WALLAC PRODUCTS

In addition to the DELFLA system the following specific reagents are available:

- |            |   |
|------------|---|
| AD0038     | Eu-labelled anti-phosphotyrosine, (PY20), 50 µg (for ten plates)  |
| AD0040     | Eu-labelled anti-phosphotyrosine, (PT66), 50 µg (for ten plates)  |
| AD0092     | Eu-labelled anti-phosphothreonine, 10 µg (for 1-2 plates)         |
| AD0097     | Eu-labelled anti phosphothreonine-proline, 10 µg (for 1-2 plates) |
| AAAND-0005 | Streptavidin-coated yellow plate, 10 plates                       |
| CC11-B10   | Streptavidin-coated white 384 plate, 10 plates                    |
| CC41-1210  | Anti-GST coated yellow plate, 10 plates                           |

## PERKINELMER LIFE SCIENCES ASSAY SERVICE

Eu-labelled anti-phosphoserine antibody service

## REFERENCES

Assays are currently routinely used in a number of drug discovery laboratories in the UK and USA. Braunwalder et al. Measurement of the protein tyrosine kinase activity of c-src using time-resolved fluorometry of europium chelates. *Anal. Biochem.* 238, 159-164 (1996). Visit [lifesciences.perkinelmer.com/referencedb](http://lifesciences.perkinelmer.com/referencedb) for more references. For more information please contact your local sales representative.



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

**European Headquarters:** PerkinElmer Life Sciences, Imperiastraat 8, B-1930 Zaventem, Belgium, +32 2 717 7911

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