

DELFI[®]A Protein Kinase assays

Introduction

Protein kinases use ATP to phosphorylate certain amino acid residues (tyrosine, serine or threonine) on their substrates. In receptor mediated signaling the phosphorylation forms an important secondary messenger system. Phosphorylation can be measured following radioactive ³²P incorporation. However, this involves the problems of radioactive work, safety and disposal. Eu-labeled anti-phosphotyrosine, -threonine and threonine-proline are available for the sensitive detection of phosphorylation levels on various substrates using time-resolved fluorometry and DELFIA[®] 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.

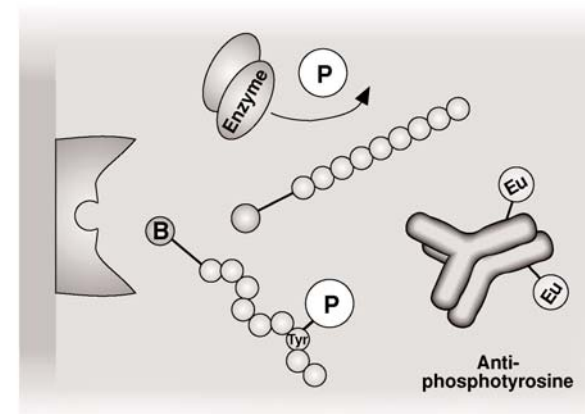


Figure 1. Detection of phosphorylation using Eu-labeled anti-phosphotyrosine and streptavidincoated microtitration plates.

3. Add Eu-(or biotin-) labeled antibodies in DELFIA 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 DELFIA Platewash (1296-026).
5. Add 200 µl of DELFIA Enhancement Solution, shake for 10 min with Plateshaker (1296-003) to guarantee a stable signal level and measure the plate with a time-resolved fluorometer, eg. the PerkinElmer[®] VICTOR or EnVision[™].

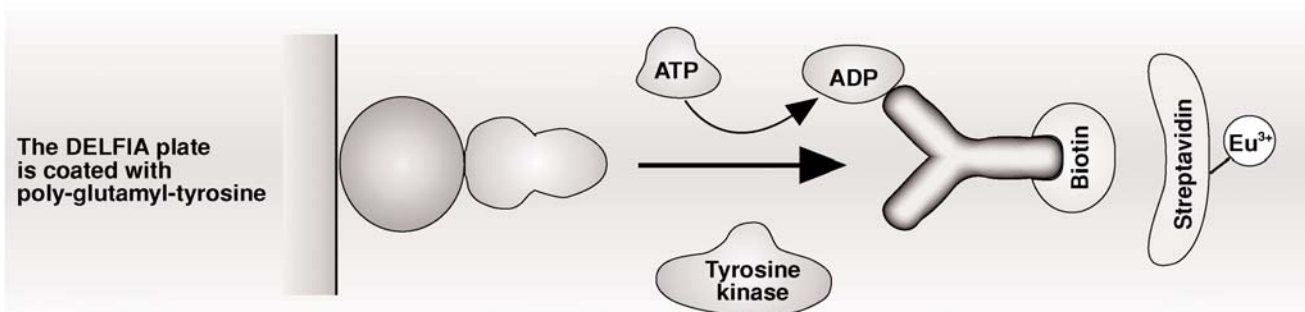


Figure 2. Schematic assay for detection of phosphorylation using Eu-labeled streptavidin for quantitation of biotinylated anti-phosphotyrosine antibody. For more information see Braunwalder *et al.*

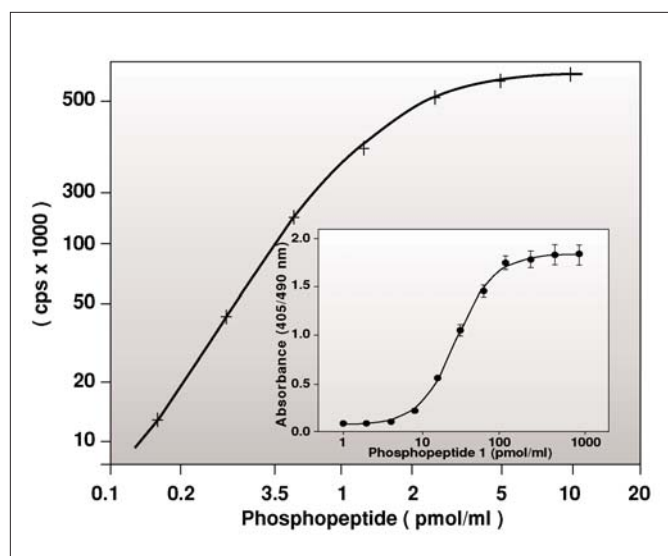


Figure 3. Quantitation of phosphorylated phosphopeptide using Eu-labeled anti-phosphotyrosine.

PerkinElmer assay services

Eu-labeled anti-phosphoserine/threonine antibody service

References

Currently assays are 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 www.perkinelmer.com/DELFI for more information or contact your local PerkinElmer Sales Representative.

Related PerkinElmer products

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

| | |
|------------|---|
| AD0038 | Eu-labeled anti-phosphotyrosine, (PY20), 50 µg (for ten plates) |
| AD0040 | Eu-labeled anti-phosphotyrosine, (PT66), 50 µg (for ten plates) |
| AD0092 | Eu-labeled anti-phosphothreonine, 10 µg (for 1-2 plates) |
| AD0159 | Eu-labeled anti-phosphotyrosine, (P-Tyr-100), 10 µg |
| AD0183 | Eu-N1 labeled anti-phospho-AKT substrated, 10 µg |
| AD0185 | Eu-N1 labeled anti-phosphoserine, 10 µg |
| AD0189 | Eu-N1 labeled anti-phospho-(Ser) 14-3-3 motif, 10 µg |
| AAAND-0005 | Streptavidin-coated yellow plate, 10 plates |
| 1244-360 | Eu-labeled streptavidin, (for 50-125 plates) |
| 1244-111 | DELFI Assay Buffer, 250 ml (for 5 plates) |
| 4002-0010 | DELFI Assay Buffer, 1,000 ml |
| 1244-114 | DELFI Wash concentrate, 25 x concentrate, 250 ml |
| 1244-105 | DELFI Enhancement Solution, 250 ml |
| 4001-0010 | DELFI Enhancement Solution, 1,000 ml |
| 1296-026 | DELFI Platewash |
| 1296-003 | DELFI Plateshake |
| 1296-014 | Multipette |
| 1296-016 | Combitips, 5 ml, pack of 100 |
| 1296-017 | Combitips, 2.5 ml, pack of 100 |

For a complete listing of PerkinElmer's time-resolved fluorescent readers, please visit our website at www.perkinelmer.com/platereaders.

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