Screening for Specific Antibodies for Ser/Thr Kinase Assays Based on Time-Resolved Fluorescence

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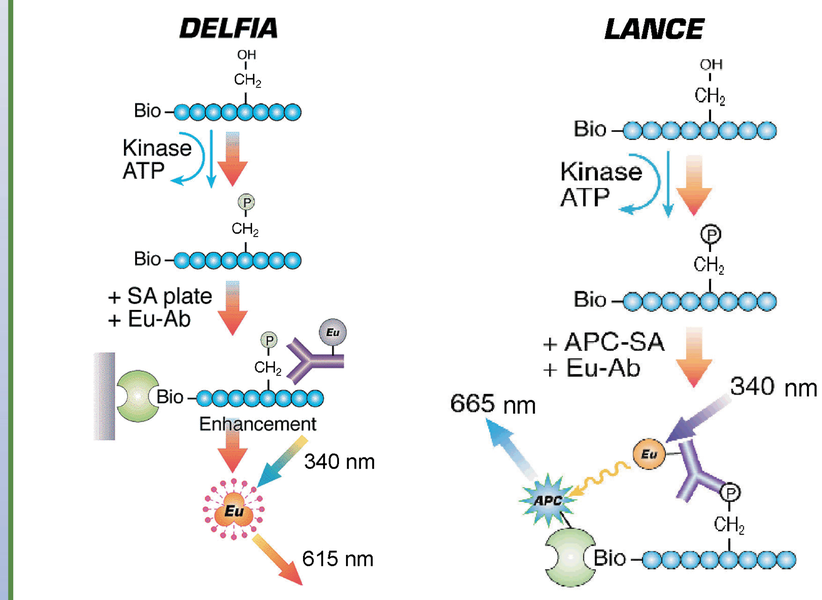
INTRODUCTION

In order to help setting up non-radioactive serine/threonine kinase assays we provide a panel of europium (Eu)-labelled antiphosphoserine/threonine antibodies that are used in the antibody specificity test service for customer supplied substrates. In the present study we have tested the recognition of seven different peptide sequences containing phosphorylated serine/threonine by seven Eu-labelled antibodies using heterogeneous DELFIA® and homogenous LANCE™ technologies.

ASSAY PRINCIPLES

DELFIA® is a heterogeneous time-resolved fluorometric assay where the enhancement step assures high sensitivity and wide response range. The assay is based on substrates immobilised on a microtitration plate. Eu-labelled antibodies are used to quantify phosphorylation.

LANCE™ kinase assay is a homogenous method based on time-resolved detection of energy transfer (TR-FRET) between a highly fluorescent Eu-chelate with a long decay-time and allophycocyanin-labelled streptavidin (SA-APC). As a simple and rapid assay, LANCE is well suited for automation and miniturisation in HTS. Both DELFIA and LANCE assays are measured using Wallac 1420 multilabel counter, VICTOR^2™V.



MATERIALS AND METHODS

The antibodies were supplied by Cell Signaling Technology and labelled with Eu-W1024 chelate (PerkinElmer Life Sciences). Substrates with 10 % phosphorylation were made by mixing corresponding phosphorylated and unphosphorylated peptides. Unphosphorylated peptide was used as a negative control.

The DELFIA assays were carried out on a streptavidin coated 384-well plate in DELFIA Assay Buffer or in DELFIA Assay Buffer supplemented with 0.1 % Tween 20. The peptides (20 nmol/L, with 10 and 0 % phosphorylation) were incubated for 1 hour (60 µL/well). After washing the Eu-labelled antibodies were added (250 ng/mL) and incubated for 1 hour. After unbound Eu-labelled antibodies were washed away, bound Eu was dissociated into the DELFIA Enhancement Solution and measured with VICTOR.

The LANCE reactions were set up in assay buffer as follows: 100 nmol/L substrate (with 10 and 0 % phosphorylation), 1 nmol/L Eu-labelled antibody and 50 nmol/L SA-APC (PerkinElmer Life Sciences). Assay buffer was 50 mmol/L Tris-HCl, pH 7.8 containing 0.9 % NaCl, 0.05 % sodium azide and 0.5 % BSA. The reaction mixtures were incubated in a white 384-well plate (60 µL/well) for 30 min and measured on VICTOR.

RESULTS

Table 1
DELFIA results

peptide sequence		signal-to-noise ratio			
bio-VKGRTW-pT-LCGTPEYL	P-PKS (Thr 197)	Eu-Ab 1, S/N = 9	Eu-Ab 2, S/N = 33	Eu-Ab 3, S/N = 9	Eu-Ab 4, S/N = 20
bio-MHRQE-pT-VDCLKKFNA	P-CamKII (Thr 286)	Eu-Ab 2, S/N = 7	Eu-Ab 3, S/N = 3	Eu-Ab 4, S/N = 20	
bio-AENFDRFF-pT-RHPPVC	P-PKCbetaII (Thr 633)	Eu-Ab 1, S/N = 106	Eu-Ab 2, S/N = 170	Eu-Ab 3, S/N = 43	Eu-Ab 4, S/N = 73
bio-CGKKRKR-pS-RKESYSI	P-H2B (Ser 32)	Eu-Ab 4, S/N = 38	Eu-Ab 5, S/N = 10		
bio-CAEYLRSI-pS-LPVPVL	P-Ask (Ser 967)	Eu-Ab 6, S/N = 289	Eu-Ab 7, S/N = 213		
bio-CGLYRSP-pS-MPENLNRPRL	cdc25 (Ser 216)	Eu-Ab 3, S/N = 5	Eu-Ab 4, S/N = 2	Eu-Ab 6, S/N = 117	Eu-Ab 7, S/N = 193
bio-RPHFPQF-pS-YSASGTC	Akt (Ser 473)	Eu-Ab 1, S/N = 138	Eu-Ab 2, S/N = 5	Eu-Ab 6, S/N = 3	Eu-Ab 7, S/N = 2

Table 2
LANCE results

peptide sequence		signal-to-noise ratio			
bio-VKGRTW-pT-LCGTPEYL	P-PKS (Thr 197)	Eu-Ab 1, S/N = 4	Eu-Ab 2, S/N = 4	Eu-Ab 3, S/N = 3	Eu-Ab, 4 S/N = 4
bio-MHRQE-pT-VDCLKKFNA	P-CamKII (Thr 286)	Eu-Ab 2, S/N = 2	Eu-Ab 4, S/N = 5		
bio-AENFDRFF-pT-RHPPVC	P-PKCbetaII (Thr 633)	Eu-Ab 1, S/N = 5	Eu-Ab 2, S/N = 6	Eu-Ab 3, S/N = 4	Eu-Ab 4, S/N = 8
bio-CGKKRKR-pS-RKESYSI	P-H2B (Ser 32)	Eu-Ab 4, S/N = 3	Eu-Ab 5, S/N = 3		
bio-CAEYLRSI-pS-LPVPVL	P-Ask (Ser 967)	Eu-Ab 6, S/N = 14	Eu-Ab 7, S/N = 5		
bio-CGLYRSP-pS-MPENLNRPRL	cdc25 (Ser 216)	Eu-Ab 3, S/N = 2		Eu-Ab 6, S/N = 26	Eu-Ab 7, S/N = 29
bio-RPHFPQF-pS-YSASGTC	Akt (Ser 473)	Eu-Ab 1, S/N = 35		Eu-Ab 6, S/N = 2	Eu-Ab 7, S/N = 2

As can be seen (tables 1 and 2) all peptide sequences were recognised by at least two antibodies and all except one of the antibodies recognises different peptide sequences proving their generic nature.

Both DELFIA and LANCE assays were set up without optimising concentrations of labelled reagents.

CONCLUSIONS

- Both LANCE and DELFIA technologies work well with this type of serine/threonine kinase assay.
- The antiphosphoserine/threonine antibody specificity test service is a simple, cost-effective and time saving way to find a specific antibody for serine/threonine kinase substrate.
- The report supplied to the customer after finalising the antibody specificity test service gives a good starting point for further assay development.
- In the present study we tested seven different Eu-labelled antiphosphoserine/threonine antibodies. The list of Eu-labelled antibodies available for the antibody specificity test service is continuously extended.