Application Notes

Miniaturization of LANCE™ Kinase Assays

INTRODUCTION

Homogeneous energy-transfer assays often demand careful optimization due to the sensitivity of the energy-transfer reaction to external interference (Hemmilä and Webb, 1997). The following observations are mainly based on experiments done with LANCE kinase assays (Figure 1) but they also apply to many other assay types.

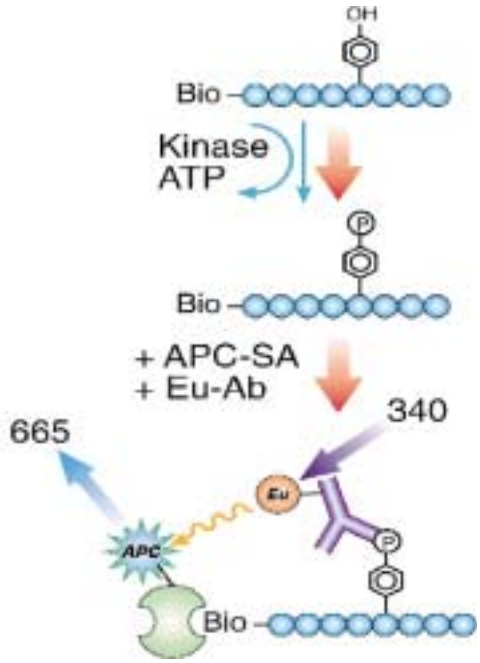

Figure1. Principle of the LANCE kinase assay (Hemmilä and Ahola, 1997)

EFFECT OF BUFFER

Many screening assays require special buffers for enzyme activity. To prevent non-specific binding, BSA (0.1- 0.5 mg/ml) or Tween 20 (0.1- 0.5%) should be added to the buffers provided that this does not disturb the enzymatic activity. Tween or BSA should be included at least in the label dilution buffer. Moreover, Brij 35 has been observed to increase signal-to-noise ratios with certain kinase-substrate-antibody combinations, which should be considered when optimizing assays.

Some metal ions, such as Mn^{2+} and Zn^{2+}, have been observed to disturb europium fluorescence. If these ions are essential for enzyme activity they should be chelated from the reaction mixture using e.g. EDTA before addition of the label mixture. The concentration of EDTA should equal the concentration of metal (for more information see the section below: How to Stop an Enzyme Reaction?)

CONCENTRATION OF LABELS

In many homogeneous high-throughput screening assays labelled streptavidin is used

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as a generic reagent (Ahola et. al. 1998). Usually, to achieve optimal sensitivity, the concentration of biotin and biotin binding sites should be equal. One streptavidin can bind up to four biotins but often three has been used as a coefficient.

In some assays very high substrate concentrations are needed and, as a consequence, the concentration of streptavidin needed to bind all of the substrate is also high. Very high concentrations of e.g. streptavidin-allophycocyanin (SA-APC) can increase the background and self-quenching of APC may also occur. This can usually be avoided by increasing the total volume of the assay in the detection step. However, in assays performed in a 1536-well plate this is not always possible due to the limited volume of the well, therefore increased background might become a problem. Such situations always need extra careful optimization between substrate concentration and assay sensitivity. The effect of SA-APC concentration on the signal-to-noise ratio of a kinase assay performed in a 1536-well plate is presented, by way of an example in figure 2.

Because the concentration of the actual analyte (e.g. phosphorylated amino acid in kinase assays) is most often low and the assays do not demand a wide linear range, the concentration of the actual detection molecule (usually antibody) can be low. In a typical kinase assay the concentration of e.g. anti-phosphotyrosine antibody can be as low as 1 nM.

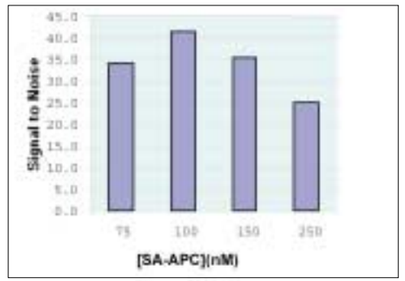

Figure 2. Effect of different concentrations of streptavidin-allophycocyanin on signal-to-noise ratios in a LANCE kinase assay performed in a 1536-well plate. Concentration of anti-phosphotyrosine-antibody was constant. SA-APC concentration should have been 250 nM to bind all biotinylated peptide but the highest signal-to-noise ratios were achieved using 100 nM SA-APC. With higher concentration of SA-APC, the decrease in S/N ratio is due to increased background and self-quenching.

EFFECT OF PLATE COLOUR AND WELL DENSITY

The colour of the plate has a significant effect both on signal levels and signal-to-noise ratios. The highest signals have been achieved using white plates. On the other hand, the highest signal-to-noise ratios have been measured using black plates. There may be some variations between different manufacturers' plates thus it is advisable to test plates from different sources for your favourite assay. Figure 3.A shows the signal-to-noise ratios with black, white and clear strip plates and figure 3.B the absolute signals from the same plates.

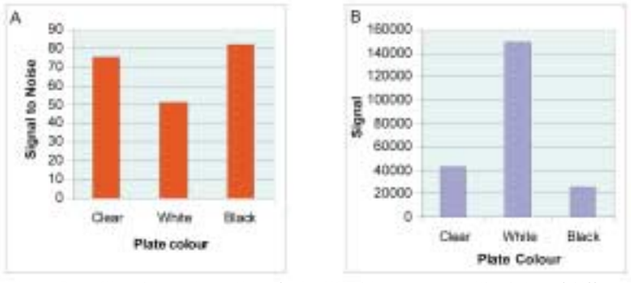

Figure 3. A: Signal-to-noise ratios of a LANCE kinase assay with plates of different colours. B: Absolute signals with the same plates

Some samples from a chemical library might disturb the energy transfer in a LANCE assay. This disturbance can be corrected by using a simple quench-correction algorithm (Hemmilä et. al., 1998) which performs most efficiently when using black plates and a small volume.

EFFECT OF MINIATURIZATION

Miniaturization can dramatically influence incubation times and assay costs (table 1). Usually the concentration of reagents needs to be increased when moving to higher well-density plates due to increased surface-to-volume ratio. The buffers need also to be optimized more carefully to minimize non-specific binding. Due to their homogeneous nature, LANCE -based assays can be easily miniaturized down to 1536-well plates and beyond without effects on the performance (Ollikka et. al., 1998). However, the use of clear plates in 1536 format in LANCE assays is not recommended due to increased well-to-well crosstalk.

	384-plate	1536-plate
Enzyme / assay	2 Units	0.8 Units
Enzyme cost / assay	~$4	~$2
Incubation time	3.5 hours	1 hour

Table 1. Effect of miniaturization on enzyme cost and incubation times. Considerable savings in terms of both time and money can be achieved.

HOW TO STOP AN ENZYME REACTION?

Many enzyme reactions need to be measured before the reaction has achieved a plateau. Some reactions can be stopped by chelating the metal needed for enzymatic activity with e.g. EDTA, but high concentrations of EDTA can release Eu^{3+} from its chelate especially during prolonged incubations. To avoid this it is preferable to use an EDTA concentration just sufficient to remove all the metal needed for enzymatic activity from the reaction mixture.

REFERENCES

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PRODUCTS AVAILABLE

CR130-100 SureLight-APC Streptavidin, 1 mg
CR130-150 SureLight-APC Streptavidin, 50 mg
AD0059 Highly fluorescent APC-SA, 1 mg
AD0066 LANCE Eu-W1024 labelled PY20 antibody, 50 µg
AD0067 LANCE Eu-W1024 labelled PY20 antibody, 1 mg
AD0068 LANCE Eu-W1024 labelled PT66 antibody, 50 µg
AD0069 LANCE Eu-W1024 labelled PT66 antibody, 1 mg
AD0094 LANCE Eu-W1024 labelled anti-phosphothreonine antibody (CST*), 10 µg
AD0095 LANCE Eu-W1024 labelled anti-phosphothreonine antibody (CST*), 1 mg
AD0099 LANCE Eu-W1024 labelled anti-phosphothreonine-proline antibody (CST*), 10 µg
AD0100 LANCE Eu-W1024 labelled anti-phosphothreonine-proline antibody (CST*), 1 mg
AD0121 LANCE Tyrosine Kinase start up reagents

AAAND-00002 White 384 well plate, 50 plts
1508-0010 Black 384-well plate, 50 plts

1420 VICTOR2 Multilabel counter, HTS model
1440 ViewLux Plate Imager

ASSAY SERVICES

- Selection of Eu-labelled anti-phospho-serine antibodies
- LANCE Kinase assay development

For more information please contact your local sales representative,
or e-mail us at assayservices@perkinelmer.com

* Antibody supplied by Cell Signaling Technology (CST).

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