Miniaturization of the Phosphodiesterase SPA assay

Peter J. Tatnell, Molly Price-Jones & Kelvin T. Hughes

Amersham Biosciences UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, England, HP7 9NA.
(Telephone: +44 (029) 2052 6417, Fax +44 (029) 2052 6474, e:mail: leadseeker@eu.amershambiosciences.com)*

INTRODUCTION

Scintillation Proximity Assay (SPA) is a robust, versatile and sensitive homogeneous screening technology. Traditionally, screening assays based upon SPA have been performed in 96-well microplates. However, with the use of increasingly large combinatorial libraries and the growing number of potential drug targets, the need to increase screening throughput whilst minimising cost is a major consideration for the pharmaceutical industry. Screening of assays performed in 1536-well microplates using the Amersham Biosciences LEADseeker™ homogeneous imaging system is an effective method to address this consideration.

This study describes the miniaturization of the phosphodiesterase (PDE) SPA to a 1536 microplate format using the LEADseeker homogeneous imaging system.

There are at least seven distinct PDE isozyme families that differ with respect to their tissue distribution, activity and substrate preferences. The cAMP-specific PDE4 has been identified as a viable drug candidate for a variety of inflammatory disorders and inhibitors (such as theophylline) have been used for the treatment of asthma.[1, 2]

PDE4 catalyses the hydrolysis of the intracellular messenger adenosine 3',5'-cyclic phosphate (cAMP) to the non-cyclic adenosine 5'-monophosphate (AMP). The SPA assay is based upon the selective interaction of the tritiated product with yttrium oxide LEADseeker beads.

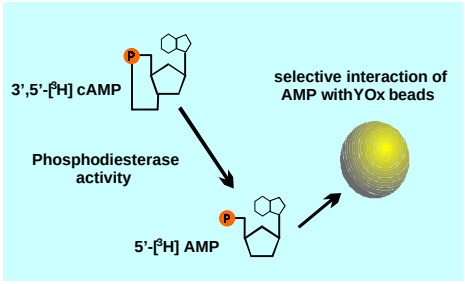

Figure 1. *Phosphodiesterase [^{3}H] cAMP SPA.*

METHOD

Assays were initiated according to the protocol contained within the phosphodiesterase [^{3}H] cAMP SPA enzyme assay (TRKQ7090, Amersham Biosciences). Briefly, this is designed for a 96-well microplate format, in which the assay contains 500ng PDE and 50nCi [^{3}H] cAMP in a volume of 100µl (0.5M Tris/HCl pH 7.5, 83mM $MgCl_2$ and 17mM EGTA). After 30min at 30°C, assays were terminated by the addition of 50µl yttrium oxide beads (20mg/ml) in the presence of 18mM $ZnSO_4$. Miniaturization from a 96-well format to 384-, 384-low volume (-LV) and 1536-well formats represents a 4-, 10- and 20-fold reduction respectively in the assay volume and reagent usage.

Assays performed in 384-well formats were imaged for 5min using the LEADseeker homogeneous imaging system, while those in 384-LV and 1536-well microplates were imaged for 10min using coincident averaging. The bovine PDE4 isoform and the isozyme non-selective inhibitor theophylline were used to perform these analyses.

RESULTS

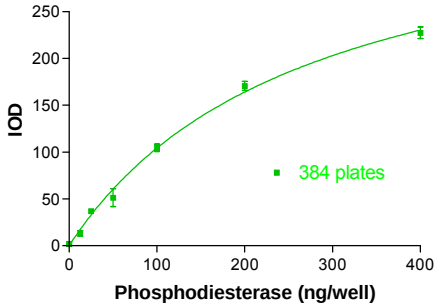

Figure 2. *Enzyme dependent hydrolysis of [^{3}H] cAMP. Performed in Greiner 384-well microplates. Values are means ! SEM (n=3). IOD = Integrated optical density values.*

Plate type	Signal (S) (+ enzyme)	Background (B) (- enzyme)	S:N ratio	Z'-factor
384	115.2 ± 5.6	13.4 ± 4.3	8.6	0.71
384-LV	114.2 ± 11.5	9.6 ± 1.9	11.9	0.62
1536	83.6 ± 6.7	7.9 ± 1.1	10.6	0.69

Table 1. *Integrated optical density values (and the signal to background ratio) detected in the presence (+) and absence (-) of phosphodiesterase.*

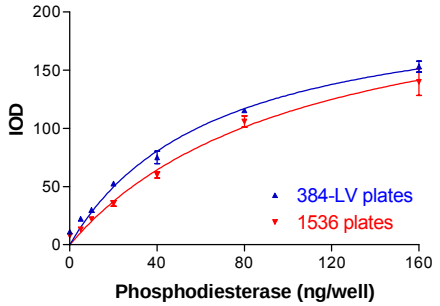

Figure 3. *Enzyme dependent hydrolysis of [^{3}H] cAMP. Performed in Greiner 384-low volume & 1536 microplates. Values are means ± SEM (n = 3).*

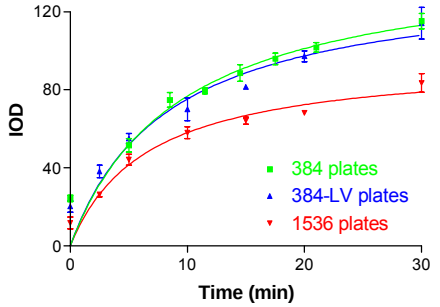

Figure 4. *Time dependent hydrolysis of [^{3}H] cAMP. Performed in Greiner 384, 384-low volume & 1536-well microplates. Values are means ± SEM (n=4).*

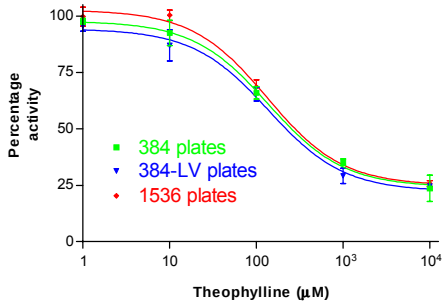

Figure 5. *Theophylline inhibition of Bovine PDE4.*

The calculated IC_{50} for the general PDE inhibitor theophylline against bovine PDE4 was ~136µM. This is comparable to that for the inhibition of the equivalent human isoform [2].

Plate type	Assay volume	[^{3}H] cAMP (nCi)	PDE (ng)	Reduction
96	100µl	50	500	n/a
384	25µl	12.5	125	4
384-LV	10µl	5	50	10
1536	5µl	2.5	25	20

Table 2. *Reduction in assay volume and components associated with the miniaturization of the phosphodiesterase SPA [^{3}H] cAMP SPA.*

Plate type	Counter/imager	Counter/imager Time	Total time
96	PMT scintillation	1min per well	~20min
384	PMT scintillation	1min per well	~60min
384-LV	PMT scintillation	1min per well	~60min
384	LEADseeker	2x5min per plate	~10min
384-LV	LEADseeker	2x10min per plate	~20min
1536	LEADseeker	2x10min per plate	~20min

Table 3. *Length of time that is typically required to monitor the phosphodiesterase [^{3}H] cAMP SPA. Coincident averaging was performed to image microplates using the LEADseeker homogeneous imaging system.*

CONCLUSION

This study describes the successful miniaturization of the phosphodiesterase [^{3}H] cAMP SPA enzyme assay to a 1536 microplate format using the LEADseeker homogeneous imaging system.

This represents:

- A 20-fold reduction in assay volume/reagents
- A significant reduction in the length of time to screen individual plates
- No decrease in signal:background ratio
- Z prime factors > 0.6

REFERENCES

1. GIEMBYCZ M.A. *Biochem. Pharmacol. 43, 2041-2051, (1992).*
2. FUJII K. *ET AL.*. *J. Pharmacol. Exp. Ther. 284, 162-169, (1998).*

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