HIGH THROUGHPUT SCREENING FOR cAMP BY SCINTILLATION PROXIMITY RADIOIMMUNOASSAY.

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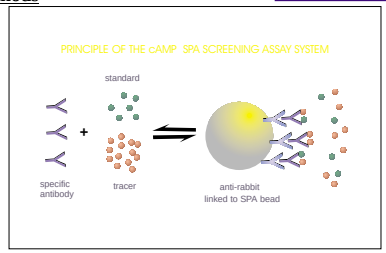
Introduction

The development of SPA (Scintillation Proximity Assay, Amersham International) has enabled the production of a one-step RIA in which radioactivity associated with antibody-bound cAMP can be counted in the presence of unbound radiolabelled cAMP without the need to separate bound from free, or the addition of a liquid scintillation cocktail.

The introduction of low-density polyvinyl toluene (PVT)-based fluomicrospheres (SPA beads) described here, remain in suspension for long periods of time. This property greatly improves pipetting accuracy and facilitates complete assay automation. The method is designed to be carried out in microplates and is optimal for estimating cAMP in large sample numbers, with little technical intervention.

Methods

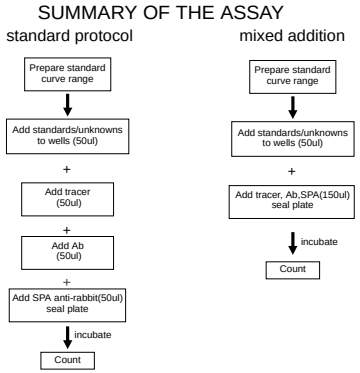


Immunogens

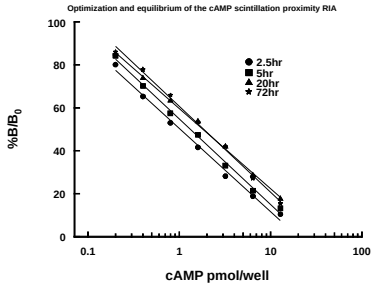
Human serum albumin: cAMP conjugates were prepared by the method of Horton *et al* (1). Antisera was raised in New Zealand white rabbits.

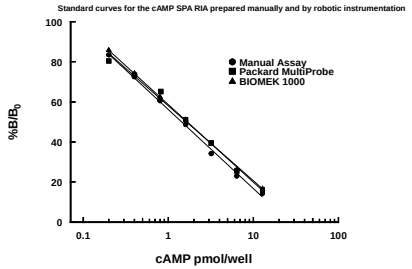
cAMP Radioiodination

cAMP was iodinated by the method of Horton and Baxendale (2).



Results

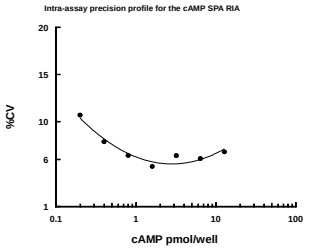




Cross reactivity data for the cAMP assay

Compound	Percent Cross Reactivity
cAMP	100
cIMP	0.4
cGMP	0.0004
cCMP	0.00005
cTMP	0.0001
AMP	0.0002
ADP	0.0001
ATP	0.00002
EDTA	0.0000001
Theophylline	0.000002
Iso-butyl-methyl-xanthine	0.000008

The specificity of the cAMP antisera was determined by the 50% displacement technique.



Sample	pmol cAMP/well +/- 1SD	%CV	n
A	0.352 +/- 0.040	11.3	20
B	0.999 +/- 0.056	5.7	20
C	4.189 +/- 0.123	2.9	20

The within-assay precision for duplicate determinations was calculated by measuring unknown samples in the assay.

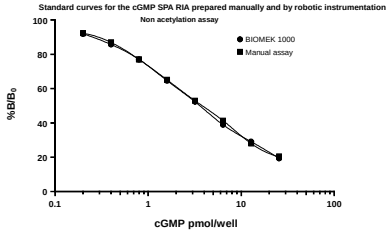
Sample	pmol cAMP/well +/- 1SD	%CV	n
A	0.331 +/- 0.036	10.8	20
B	0.884 +/- 0.099	11.2	20
C	3.946 +/- 0.355	9.0	20

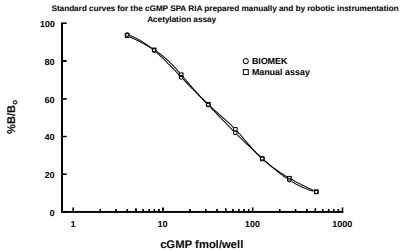
Between-assay reproducibility was assessed by sequential measurement of samples in different assays.

- Assay Sensitivity: 0.1pmol/well (0.025pmol/well: high sensitivity protocol).

cGMP SPA Screening Assay System

- Dual Range Method - includes ultra - high sensitivity option.

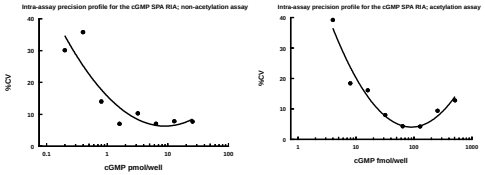




Cross-reactivity data for the cGMP Assay

Compound	Cross Reactivity Percent	
	Non-acetylation	Acetylation
cGMP	100	100
cAMP	0.0049	0.0074
AMP	< 0.00044	< 0.0000054
ADP	< 0.00044	< 0.0000054
ATP	< 0.00044	< 0.0000054
GMP	0.2	0.00096
GDP	0.09	0.00048
GTP	0.04	0.00016

The specificity of the cGMP antisera was determined by the 50% displacement technique



- Assay Sensitivity: 0.2pmol/well: non acetylation protocol. 4fmol/well: acetylation protocol.

Discussion

The new cAMP and cGMP SPA screening assays offer:

- High sample throughput and assay precision due to the combination of homogeneous SPA technology in a microtitre plate format.
- Exceptional specificity allowing confidence in results in the presence of closely related compounds.
- Complete assay automation through the use of second generation PVT SPA beads (which do not require agitation during incubation) and the utilisation of microtitre plate liquid scintillation counters.
- Reduced cost and hazard - no need for liquid scintillant.

References

1. Horton, J.K, Martin, R.C, Kalinka, S., Cushing, A., Kitcher, J.P., O'Sullivan, M.J., and Baxendale, P.M. (1992) Enzyme immunoassays for the estimation of adenosine 3',5'-cyclic monophosphate and guanosine 3',5'-cyclic monophosphate in biological fluids. J. Immunol Methods 155, 31-40.
2. Horton, J.K., and Baxendale, P.M. (1995) Mass measurements of cyclic AMP formation by radioimmunoassay, enzyme immunoassay and scintillation proximity assay. In: Methods in Molecular Biology, Vol 41: Signal Transduction Protocols, Kendall, D.A., and Hill, S.J. (Eds) pp 91-105. Humana Press , Totowa, N.J.

- **cAMP SPA Screening Assay System RPA556**
- **cGMP SPA Screening Assay System RPA557**

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