PerkinElmer™
life sciences.

Comparison of cAMP Assay Technologies for High Throughput Screening

Patricia Kasila and Harry Harney

PerkinElmer Life Sciences, 549 Albany Street, Boston, MA 02118

1

Abstract

Homogeneous cAMP assays have been developed to allow for the direct measurement of receptor mediated adenylyl cyclase activation/inhibition in G-protein coupled receptor systems. PerkinElmer has developed three homogenous cAMP assays using different technologies. These assays can all be easily modified to accommodate many robotic systems and situations in fully homogeneous assays. The platforms described here include scintillation proximity technology using the Adenylyl Cyclase Activation FlashPlate kit (Cat # SMP701), fluorescence polarization using [FP]^2 cAMP kit (Cat # FPA202), and luminescent proximity technology using the AlphaScreen cAMP assay (Cat # 6760600C). Studies were performed to compare sensitivity, precision, and ease of use of each assay. Requirements for technology attributes vary greatly depending on the individual user. The comparative data shown here will highlight advantages of each technology.

2 Introduction

cAMP Technology Comparison

- Adenylyl Cyclase Activation FlashPlate Assay
- [FP]² cAMP Assay
- AlphaScreen cAMP Assay

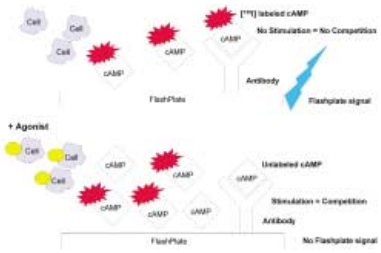
Comparisons

Using whole cells with overexpressed β2 Adrenergic receptors

- Standard Curve Dynamic Range
- Forskolin Dose Response
- Agonist Dose Response
- Antagonist Dose Response

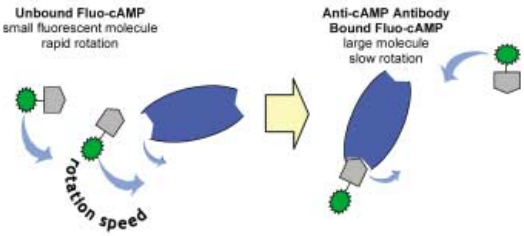
3 Basic Principle

Adenylyl Cyclase Activation FlashPlate Assay



- agonist-stimulated cAMP displaces [^{125}I] labeled cAMP from Antibody

[FP]² Fluorescence Polarization cAMP Assay

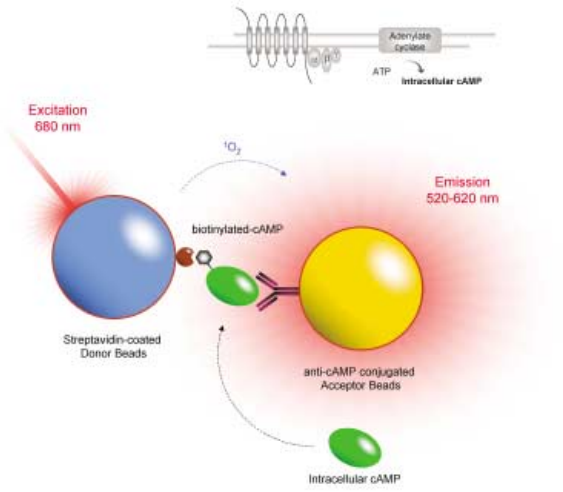


- agonist-stimulated cAMP displaces Fluo-cAMP from Antibody produces lower polarization signal

4

Basic Principle (cont'd)

AlphaScreen cAMP



- agonist-stimulated cAMP displaces biotinylated cAMP from anti-cAMP immobilized on acceptor beads.

5

Kit Components

Adenylyl Cyclase Activation FlashPlate Assay (PerkinElmer Catalog # SMP701)

- cAMP Standard
- Stimulation Buffer
- [^{125}I]cAMP Tracer
- Detection Buffer
- Adenyly Cyclase FlashPlates

[FP]^2 Fluorescence Polarization cAMP Assay (PerkinElmer Catalog # FPA202)

- cAMP Standard
- Stimulation Buffer
- cAMP Antibody
- Fluo-cAMP Tracer
- Detection Buffer
- Microtiter plates

AlphaScreen cAMP Assay (PerkinElmer Catalog # 6760600C)

- Biotinylated cAMP
- Streptavidin Donor Beads
- Anti-cAMP Acceptor Beads

6 Assay Protocols

Adenylyl Cyclase Activation FlashPlate Assay

1- 10 µL of cAMP standards or cells diluted in Stimulation Buffer

2- 15 µL PBS (standards) or forskolin or agonist/antagonist (cells)

3- Incubate for 30 – 60 min. at room temperature

4- 25 µL of Detection Mix containing Detection Buffer and [^{125}I]cAMP

5- Incubate for 2 hours at room temperature

6- Read on a MicroBeta® or TopCount NXT® Microplate Scintillation Counter

[FP]^2 cAMP

1- 10 µL of cAMP standards or cells diluted in Stimulation Buffer

2- 10 µL PBS (standards) or forskolin or agonist/antagonist (cells)

3- Incubate for 30 – 60 min. at room temperature

4- 10 µL of Detection Mix containing Detection Buffer and Fluo-cAMP (5 nM final)

5- 10 µL of Anti-cAMP diluted in Detection Buffer

6- Incubate for 60 minutes at room temperature

7- Read on a Fluorescence Polarization Reader (EnVision™, ViewLux™, Victor^{2}V™ or Fusion™)

AlphaScreen cAMP

1- 5 µL cells/anti-cAMP Acceptor Beads or anti-cAMP Acceptor Beads only (standards)

2- 5 µL forskolin or agonist/antagonist or cAMP standards

3- Incubate for 30 – 60 min. at room temperature

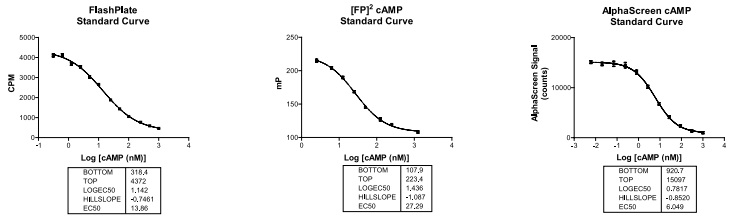
4- 15 µL of biotinylated-cAMP/Streptavidin Donor Beads Detection Mix

5- Incubate for at least 60 min. at room temperature

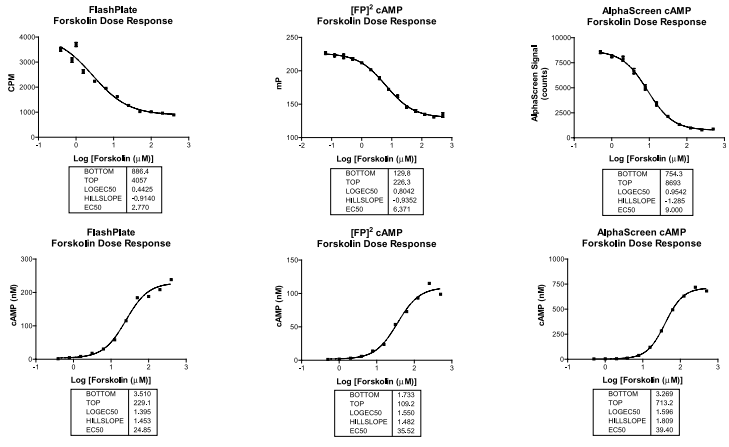
6- Read on a Fusion™-α or AlphaQuest™-HTS microplate analyzer

7 Results

cAMP Standard Curves

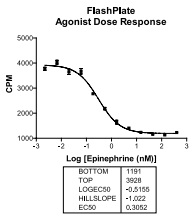


Forskolin Dose Response

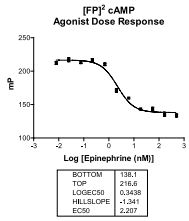


8 Results (cont'd)

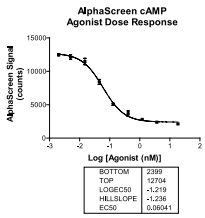
Agonist Dose Response



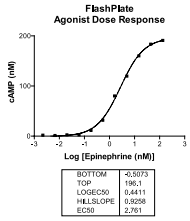
BOTTOM	1191
TOP	3928
LOGEC50	-0,5155
HILLSLOPE	-1,022
EC50	0,3052



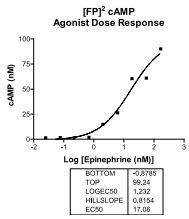
BOTTOM	138,1
TOP	216,6
LOGEC50	0,3438
HILLSLOPE	-1,341
EC50	2,207



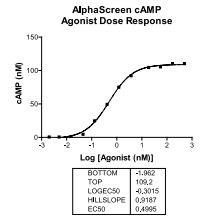
BOTTOM	2399
TOP	12704
LOGEC50	-1,219
HILLSLOPE	-1,236
EC50	0,06041



BOTTOM	-0,5073
TOP	196,1
LOGEC50	0,4411
HILLSLOPE	0,9258
EC50	2,761

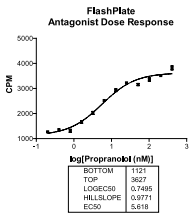


BOTTOM	-0,8765
TOP	99,24
LOGEC50	1,232
HILLSLOPE	0,8154
EC50	17,06

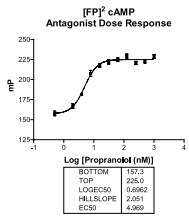


BOTTOM	-1,962
TOP	109,2
LOGEC50	-0,3015
HILLSLOPE	0,9187
EC50	0,4995

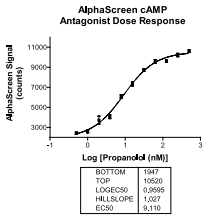
Antagonist (Propranolol) Dose Response



BOTTOM	1121
TOP	3627
LOGEC50	0,7495
HILLSLOPE	0,9771
EC50	5,618

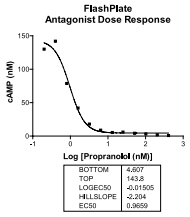


BOTTOM	157,3
TOP	225,0
LOGEC50	0,6962
HILLSLOPE	2,051
EC50	4,969

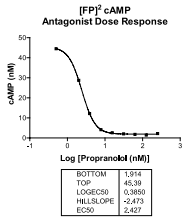


BOTTOM	1947
TOP	10520
LOGEC50	0,9695
HILLSLOPE	1,027
EC50	9,110

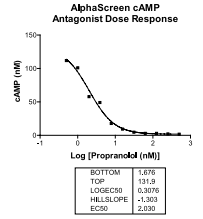
Antagonist (Propranolol) Dose Response (cont'd)



BOTTOM	4,607
TOP	143,8
LOGEC50	-0,01505
HILLSLOPE	-2,204
EC50	0,9659

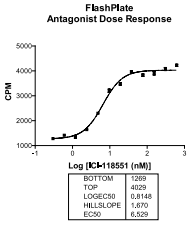


BOTTOM	1,914
TOP	45,39
LOGEC50	0,3850
HILLSLOPE	-2,473
EC50	2,427

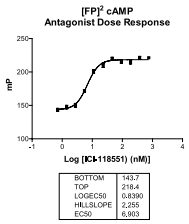


BOTTOM	1,676
TOP	131,9
LOGEC50	0,3076
HILLSLOPE	-1,303
EC50	2,030

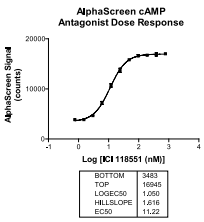
Antagonist (ICI-11885) Dose Response



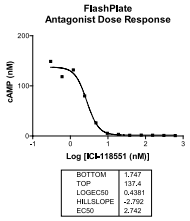
BOTTOM	1269
TOP	4029
LOGEC50	0,8148
HILLSLOPE	1,670
EC50	6,529



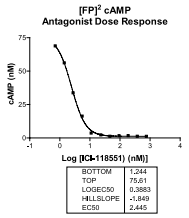
BOTTOM	143,7
TOP	218,4
LOGEC50	0,8300
HILLSLOPE	2,255
EC50	6,903



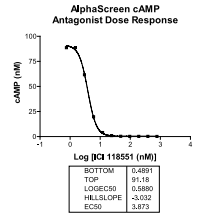
BOTTOM	3483
TOP	16945
LOGEC50	1,050
HILLSLOPE	1,616
EC50	11,22



BOTTOM	1,747
TOP	137,4
LOGEC50	0,4381
HILLSLOPE	-2,792
EC50	2,742



BOTTOM	1,244
TOP	75,61
LOGEC50	0,3883
HILLSLOPE	-1,849
EC50	2,445



BOTTOM	0,4891
TOP	91,18
LOGEC50	0,5880
HILLSLOPE	-3,032
EC50	3,873

8

Results (cont'd)

EC_{50} and Z' Comparison (Raw Data)

	Standard Curve		Forskolin Dose Response		Agonist Dose Response		Antagonist Dose Response (Propranolol)		Antagonist Dose Response (ICI-11885)	
	EC_{50}	Z'	EC_{50}	Z'	EC_{50}	Z'	EC_{50}	Z'	EC_{50}	Z'
FlashPlate	13.9	0.98	2.8	0.97	0.31	0.94	5.6	0.84	6.5	0.92
$[FP]^2$	27.3	0.87	6.4	0.74	2.2	0.80	5.0	0.76	6.9	0.87
AlphaScreen	6.0	0.77	9.0	0.70	0.06	0.89	9.1	0.86	11.2	0.84

EC_{50} of Interpolated Results

	Forskolin Dose Response	Agonist Dose Response	Antagonist Dose Response (Propranolol)	Antagonist Dose Response (ICI-11885)
FlashPlate [cAMP(nM)]	24.8	2.8	0.97	2.7
$[FP]^2$ [cAMP(nM)]	35.5	17.1	2.4	2.4
AlphaScreen [cAMP(nM)]	39.4	0.50	2.0	3.9

9

Conclusions

The cAMP technologies presented here are homogeneous assays that are highly suited for High Throughput Screening. The Adenylyl Cyclase FlashPlate Assay has been used in many HTS screening labs and has been found to be the "gold standard" for cAMP detection. $[FP]^2$ cAMP fluorescence polarization assay is a non-rad homogenous platform that is easy to use, and gives the flexibility to format the protocol depending on the needs of the user. This assay does not have the dynamic range or sensitivity of the other technologies but can be used successfully for many receptors. AlphaScreen cAMP has the widest dynamic range, excellent precision characteristics and is a valid non-rad alternative to the "gold standard" Adenylyl Cyclase FlashPlate assay.

Worldwide Headquarters: PerkinElmer Life Sciences, Inc., 549 Albany Street, Boston, MA 02118-2512 USA (800) 551-2121

European Headquarters: PerkinElmer Life Sciences, Inc., Imperiastraat 8, BE-1930 Zaventem Belgium

Technical Support: in Europe: techsupport.europe@perkinelmer.com in US and Rest of World: techsupport@perkinelmer.com

Belgium: Tel: 0800 94 540 • **France:** Tel: 0800 90 77 62 • **Netherlands:** Tel: 0800 02 23 042 • **Germany:** Tel: 0800 1 81 00 32 • **United Kingdom:** Tel: 0800 89 60 46

Switzerland: Tel: 0800 55 50 27 • **Italy:** Tel: 800 79 03 10 • **Sweden:** Tel: 020 79 07 35 • **Norway:** Tel: 800 11 947 • **Denmark:** Tel: 80 88 3477 • **Spain:** Tel: 900 973 255

S4083 09/02

www.perkinelmer.com/lifesciences