LANCE™ cAMP + Evolution™ P³ + ViewLux™ = uHTS

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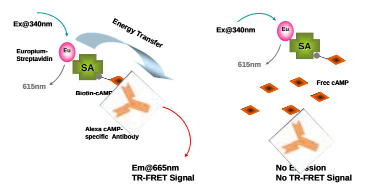
1 Abstract

High Throughput Screening Labs have been successfully automating assays to achieve higher throughput. To date only a small number of labs have been successfully able to go to even higher density formats, i.e. 1536-well microplates, due to the lack of precise liquid handling systems, homogeneous robust detection assays and high density detection equipment. Examples shown here will demonstrate that using the LANCE™ cAMP assay which allows for the direct measurement of receptor mediated adenylyl cyclase activation/inhibition in G-protein coupled receptors, the Evolution™ P³ liquid handing system and the ViewLux™ Detection Instrument, both 384-well and 1536-well formats will give comparable results with excellent precision.

2 Schematic Representation of LANCE cAMP Assay



3 Instrumentation

ViewLux™ enables you to enjoy the high throughput advantages of miniaturization. It accepts all formats including 384- and 1536-well, and sample capacity using the latter is almost 100,000 samples in one loading.

- Fluorescence intensity
- Fluorescence polarization
- Time-resolved fluorescence (TRF)
- Luminescence
- Absorbance

The Evolution™ P³ (EP³) Precision Pipetting Platform is a flexible automated liquid handler for low-to-high throughput microplate applications using 96 or 384-channel disposable tip dispense heads.

4 Materials

- LANCE™ cAMP Assay (PerkinElmer Catalog # AD0262)
  - The kit includes the following components:
    - Biotinylated-cAMP
    - Streptavidin Europium
    - Alexa 647 Labeled Antibody
    - cAMP Standard
    - Detection Buffer
- Stimulation Buffer – 1X HBSS containing 5 mM HEPES buffer, 500 µM IBMX and 0.01% BSA (PerkinElmer Catalog #CR94-100), pH 7.4
- OptiPlate - 384-well white plate (PerkinElmer Catalog # 6007290), 1536-well white plate (PerkinElmer Catalog # 6005228)
- Cells – β2 adrenergic receptor cell line (PerkinElmer Catalog # MCL-501), CHO-h5HT$_{1A}$ (PerkinElmer Catalog # MCL-508)
- Chemicals-Forskolin (Sigma# 6886)

Agonists (Epinephrine Sigma# E4250), (8OH-DPAT Sigma# H140)
Antagonists(Propanolol Sigma# P-0884) (Spiperone Sigma# S-7395)

5 Methods

- 384-well (All dispensing was done using the EP³)
  - 6 µL of cAMP standards, forskolin, agonist or antagonist in PBS
  - 6 µL cells diluted in Stimulation Buffer (3000 cells/well)containing cAMP antibody or Stimulation Buffer containing cAMP antibody without cells added to the wells containing standards
  - Incubate for 30 minutes at room temperature
  - 12 µL of Detection Buffer containing streptavidin-europium and biotinylated cAMP
  - Incubate for 60 minutes at room temperature
  - Read on a ViewLux™

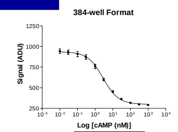
6 Methods (cont.)

- 1536-well (All dispensing was done using the EP³)
  - 2 µL of cAMP standards, forskolin, agonist or antagonist in PBS
  - 2 µL cells diluted in Stimulation Buffer (1000 cells/well) containing cAMP antibody or Stimulation Buffer containing cAMP antibody without cells added to the wells containing standards
  - Incubate for 30 minutes at room temperature
  - 4 µL of Detection Buffer containing streptavidin-europium and biotinylated cAMP
  - Incubate for 60 minutes at room temperature
  - Read on a ViewLux™

7 Results

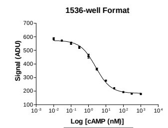
cAMP Standard Curves

384-well Format



BOTTOM	936.7
TOP	298.3
LOGEC50	0.4443
EC50	2.781

1536-well Format



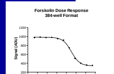
BOTTOM	573.7
TOP	183.9
LOGEC50	0.4073
EC50	2.555

Stimulation buffer containing Alexa 647-labeled anti-cAMP antibody was incubated with a series of cAMP dilutions for 30 minutes at room temperature. Detection buffer containing biotinylated cAMP and Streptavidin-Europium was added and incubated for 60 minutes at room temperature. The results were measured on a ViewLux™.

8 Results (cont.)

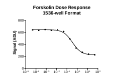
Human β2 Adrenergic Receptor

Forskolin Dose Response 384-well Format



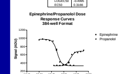
BOTTOM	983.8
TOP	341.8
LOGEC50	-0.4995
EC50	0.3166

Forskolin Dose Response 1536-well Format



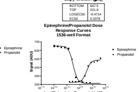
BOTTOM	647.5
TOP	221.9
LOGEC50	-0.4714
EC50	0.3378

Epinephrine/Propanolol Dose Response Curves 384-well Format



	Epinephrine	Propanolol
BOTTOM	975.4	329.9
TOP	332.3	688.2
LOGEC50	-1.357	1.315
EC50	0.04394	20.67

Epinephrine/Propanolol Dose Response Curves 1536-well Format



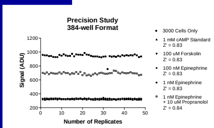
	Epinephrine	Propanolol
BOTTOM	650.0	225.6
TOP	213.3	486.7
LOGEC50	-1.307	1.310
EC50	0.04931	20.41

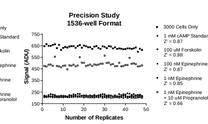
β2-adrenergic cells suspended in Stimulation buffer containing antibody were incubated with forskolin for 30 minutes at room temperature. Detection buffer containing streptavidin europium and biotinylated cAMP was added and incubated for 60 minutes at room temperature. The results were measured on a ViewLux™.

β2-adrenergic cells suspended in Stimulation buffer containing antibody were incubated with a series of agonist or 1 nM epinephrine and a series of antagonist dilutions for 30 minutes at room temperature. Detection buffer containing streptavidin europium and biotinylated cAMP was added and incubated for 60 minutes at room temperature. The results were measured on a ViewLux™.

9 Results (cont.)

Human β2 Adrenergic Receptor



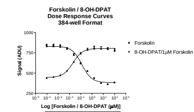


Multiple replicates were run under each condition in both 384- and 1536-well formats. Z’ calculations were performed.

10 Results (cont.)

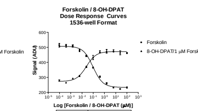
CHO-h5HT$_{1A}$ Receptor

Forskolin / 8-OH-DPAT Dose Response Curves 384-well Format



	Curve 1	Curve 2
BOTTOM	831.0	444.5
TOP	381.7	805.3
LOGEC50	-0.9026	-1.695
EC50	0.1252	0.02018

Forskolin / 8-OH-DPAT Dose Response Curves 1536-well Format



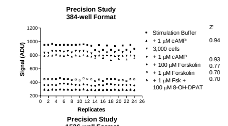
	Curve 1	Curve 2
BOTTOM	507.5	273.1
TOP	233.7	472.4
LOGEC50	-1.020	-1.610
EC50	0.09548	0.02452

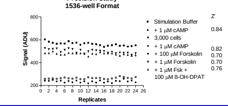
CHO-5HT$_{1A}$ cells suspended in Stimulation buffer containing antibody were incubated with forskolin (curve 1) and forskolin (1 µM) and agonist dilutions (curve 2) for 30 minutes at room temperature. Detection buffer containing streptavidin europium and biotinylated cAMP was added and incubated for 60 minutes at room temperature. The results were measured on a ViewLux™.

11 Results (cont.)

CHO-h5HT$_{1A}$ Receptor

Multiple replicates were run under each condition in both 384- and 1536-well formats. Z’ calculations were performed.





12 Conclusions

- Homogenous LANCE cAMP assay which has high sensitivity and high precision in both 384- and 1536-well formats
- Easy to miniaturize with comparable results in all formats
- Easy to fully automate using the EP³ and ViewLux™
- Ultra High Throughput screening capability

Acknowledgement for the development of the LANCE cAMP assay:

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