LANCE™ cAMP – A uHTS cAMP detection assay for use with membranes

Patricia Kasila, Hao Xie

PerkinElmer Life and Analytical Sciences, Boston MA, USA

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Abstract

Homogeneous cAMP assays have been developed to allow the direct measurement of receptor mediated adenylyl cyclase activation/inhibition in G-protein coupled receptors. Not only does homogeneous assays make it easier for screening labs to increase throughput but assays that allow the use of membranes instead of whole cells makes screening labs become much more flexible due to the elimination for the need of daily tissue culture. The LANCE™ cAMP assay is a time resolved fluorescence (TRF) assay which exhibits low background and high signal-to-noise ratios, two attributes critical for robust HTS assays. The principle of the assay involves the loss of energy transfer as the quantity of cell-derived cAMP increases, thereby decreasing the amount of biotinylated cAMP available to bind to the anti-cAMP antibody. Data presented here will demonstrate that valid results can be obtained using membranes instead of whole cells.

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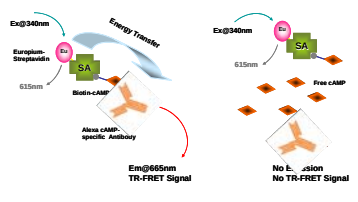
Schematic Representation of LANCE cAMP Assay



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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
- ATP Regeneration Buffer – PBS, 10 mM MgCl2, 10 mM phosphocreatine, 10 units/mL creatine phosphokinase, 10 μM GTP, 200 μM ATP, 500 μM IBMX, pH 7.4
- Detection Buffer – HEPES Buffer containing 10 mM calcium chloride and 0.35 % Triton X-100, pH 7.4
- Stimulation Buffer - PBS containing 0.1% BSA and 500 μM IBMX, pH 7.4
- OptiPlate (384-well white plate) (PerkinElmer Catalog # 6007290)
- Membranes – β2 Adrenergic receptor membrane (PerkinElmer Catalog # RBHBE2M), Human Melanocortin 4 receptor membrane (PerkinElmer Catalog # RBHMC4M)
- Chemicals-Forskolin (Sigma# 6886)

Agonists (Epinephrine Sigma# E4250, NDP-αMSH Bachem# H1075)
Antagonists(Propanolol Sigma# P-0884, SH/9119 Sigma# M4603)

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Methods

1. *Standard Curve*
   - 6 μL of cAMP standards diluted in ATP Regeneration Buffer
   - 6 μL of stimulation buffer containing Alexa 647 labeled anti-cAMP antibody
   - Incubate for 30 minutes at room temperature
   - 12 μL of Detection Mix containing biotin-cAMP and SA-Europium
   - Read on a Multilabel Plate Reader

2. *Forskolin or Agonist Dose Curve*
   - 6 μL of agonist/forskolin dilutions diluted in ATP Regeneration Buffer
   - 6 μL of membranes suspended in stimulation buffer containing Alexa 647 labeled antibody
   - Incubate 30 min. at room temperature
   - 12 μL of Detection Mix containing biotin-cAMP and SA-Europium
   - Incubate for 60 minutes at room temperature
   - Read on a Multilabel Plate Reader

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Methods (cont.)

3. *Antagonist Dose Curve*
   - 6 μL of agonist (concentration chosen to the EC_{80} from dose response curve of agonist)/antagonist dilutions diluted in ATP Regeneration Buffer
   - 6 μL of membranes suspended in stimulation buffer containing Alexa 647 labeled anti-cAMP antibody
   - Incubate 30 min. at room temperature
   - 12 μL of Detection Mix containing biotin-cAMP and SA-Europium
   - Incubate for 60 minutes at room temperature
   - Read on a Multilabel Plate Reader

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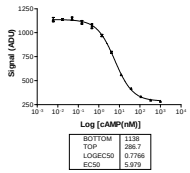
Results

cAMP Standard Curve



BOTTOM	1138
TOP	286.7
LOGEC50	0.7766
EC50	5.979

Stimulation buffer (6 μL) containing Alexa 647-labeled anti-cAMP antibody was incubated with a series of cAMP dilutions (6 μL) for 30 minutes at room temperature. 12 μl Detection buffer containing biotinylated cAMP and Streptavidin-Europium was added and incubated for 60 minutes. The results were measured on a Multilabel plate reader.

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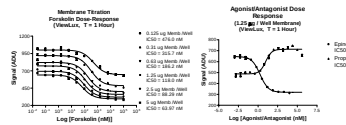
Results (cont.)

Human β2 Adrenergic Receptor



Forskolin dose response curves were performed to select the optimal membrane concentration. The optimal membrane concentration was then used to generate agonist/antagonist does response curves.

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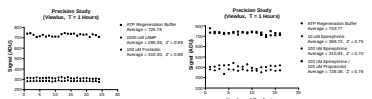
Results (cont.)

Human β2 Adrenergic Receptor



A precision study was done by running multiple replicates with different concentrations of cAMP, forskolin, agonist and antagonist.

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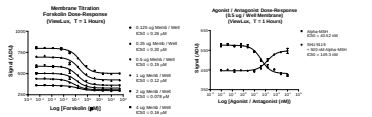
Results (cont.)

Human Melanocortin 4 Receptor



Forskolin dose response curves were performed to select the optimal membrane concentration. The optimal membrane concentration was then used to generate agonist/antagonist does response curves.

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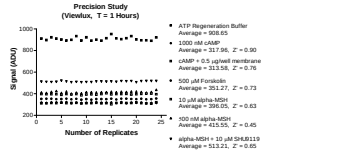
Results (cont.)

Human Melanocortin 4 Receptor



A precision study was done by running multiple replicates with different concentrations of cAMP, forskolin, agonist and antagonist.

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Instrumentation

ViewLux™

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

EnVision™

EnVision™ has the same measurement capabilities as the ViewLux but with the additional capability of measuring AlphaScreen™ technology. EnVision has a modular design allowing optimization for specific applications and fast measurement of all plate formats.

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Conclusions

- **Homogenous, easy-to-use assay**
- **The same kit can be used for membranes and whole cells**
- **LANCE cAMP assay yields rapid and reproducible data with membranes**
- **Minimizes the need for continuous cell culture**