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

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Abstract
Homogenous cAMP assays have been developed to allow the direct measurement of receptor-mediated adenyl cyclase activation/inhibition in G-protein coupled receptors. Not only does homogeneous assays make it a center for screening hits to increase throughput but assays that allow use of membranes instead of whole cells make screening hits 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 and is capable for use with robust HTS assays. The principle of the assay is the loss of energy transfer is the quantity of cell-derived cAMP available to bind to the anti-cAMP antibody. This has proven here will demonstrate that valid results can be obtained using membranes instead of whole cells.

Methods

1. Standard Curve
   - 50 µL of membranes suspended in stimulation buffer containing Alexa 647 labeled antibody
   - 200 nM cAMP
   - 750 nM cAMP
   - 3 uM cAMP
   - 10 uM Epinephrine
   - 100 uM Forskolin
   - 100 uM Propranolol
   - 10 uM Norepinephrine
   - 100 uM Salbutamol
   - 100 uM ISO

2. Assay Protocol
   - 6 µL of membranes suspended in stimulation buffer containing Alexa 647 labeled antibody
   - 100 nM cAMP
   - 300 nM cAMP
   - 1000 nM cAMP
   - 1000 nM alpha-MSH (Bachem# H1075)

3. Membrane Titration
   - 5 µg/Well
   - 10 µg/Well
   - 20 µg/Well
   - 50 µg/Well
   - 100 µg/Well

4. Results (cont.)
   - Human β2 Adrenergic Receptor
     - EC50 = 0.08
     - Z’ = 0.78
   - Human β1 Adrenergic Receptor
     - EC50 = 0.10
     - Z’ = 0.82
   - Human β3 Adrenergic Receptor
     - EC50 = 0.12
     - Z’ = 0.85

5. Results (cont.)
   - Human β2 Adrenergic Receptor
     - EC50 = 0.08
     - Z’ = 0.78
   - Human β1 Adrenergic Receptor
     - EC50 = 0.10
     - Z’ = 0.82
   - Human β3 Adrenergic Receptor
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6. Results (cont.)
   - Human β2 Adrenergic Receptor
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   - Human β1 Adrenergic Receptor
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   - Human β3 Adrenergic Receptor
     - EC50 = 0.12
     - Z’ = 0.85

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

Schematic Representation of LANCE cAMP Assay

Visualization and Analysis

Instrumentation
- ViewLux™ enables you to enjoy the high throughput screening performance of LANCE™ cAMP assay. Homogenous, easy-to-use assay that allows the use of membranes instead of whole cells.

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