

# Cell based assays in 1536-well formats – Homogenous, highly sensitive cAMP detection systems

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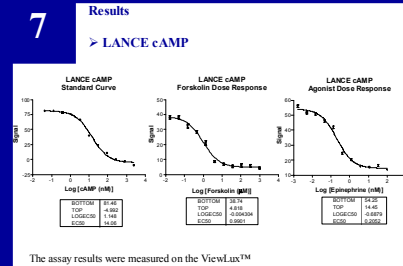
**1 Abstract**

Cell based competitive binding assays to measure cell-derived cAMP have been developed and miniaturized into 1536-well formats. All three formats display excellent performance. AlphaScreen™ cAMP assay is a bead-based, non-radioactive Amplified Luminescent Proximity Homogeneous Assay. When a biological interaction brings the beads together, a cascade of chemical reactions occur which produces a greatly amplified signal. LANCE™ cAMP is a homogeneous TRF assay where upon binding of the antibody to the cAMP, energy is transferred from the europium labeled antibody to an acceptor, allophycocyanin (APC), which is directly labeled to cAMP. [FPF] cAMPfire™ is a fluorescence polarization assay which measures the parallel and perpendicular components of fluorescence emission using plane polarized excitation. Polarization values (measured in mP units) are inversely related to the speed of molecular rotation that complex. Data presented here will demonstrate the sensitivity and precise results obtained with whole cell cAMP assays in 1536-well formats.

**4 Methods**

➤ **LANCE™ cAMP**

- 2  $\mu$ L of cAMP standards, forskolin or agonist in PBS
- 2  $\mu$ L cells diluted in Stimulation Buffer containing cAMP antibody or Stimulation Buffer containing cAMP antibody without cells added to the wells containing standards
- Incubate for 30 – 60 minutes at room temperature
- 4  $\mu$ L of Detection Buffer containing APC-cAMP
- Incubate for 60 minutes at room temperature
- Read on an appropriate reader



**10 Instrumentation**

**ViewLux™**

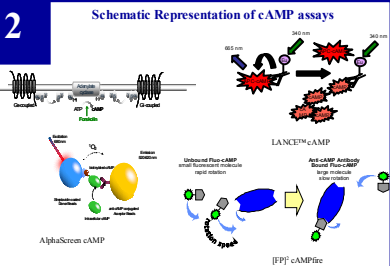
**EnVision™**

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

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.

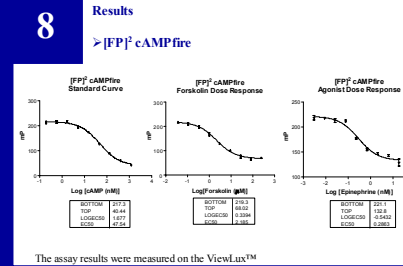
Other Detectors that were used in this study were the Fusion™, and the Victor2™.



**5 Methods**

➤ **[FPF] cAMPfire**

- 2  $\mu$ L of cAMP standards, forskolin or agonist in PBS
- 2  $\mu$ L cells diluted in Stimulation Buffer containing cAMP antibody or Stimulation Buffer containing cAMP antibody without cells added to the wells containing standards
- Incubate for 30 – 60 minutes at room temperature
- 4  $\mu$ L of Detection Buffer containing AlexaFluor™549-cAMP
- Incubate for 60 minutes at room temperature
- Read on an appropriate reader



**11 Automation**

Data shown in previous slides were generated using manual dispensing methods. An additional study was done using the Evolution™ P3 Precision Pipetting Platform.

The Evolution™ P3 is equipped with a P30 (384-well) head and P30 tips.

**3 Materials**

➤ **LANCE cAMP Assay (PerkinElmer Custom Offering)\***

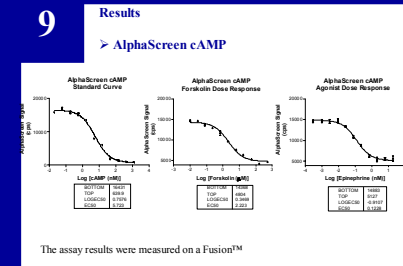
- The kit includes the following components:
  - APC-cAMP
  - Europium Labeled Antibody
  - cAMP Standard
  - Stimulation Buffer
  - Detection Buffer
- **[FPF] cAMPfire Assay (PerkinElmer Catalog# FPA203)**
- The kit includes the following components:
  - Alexa Fluor™549-cAMP
  - cAMP Antibody
  - cAMP Standard
  - Stimulation Buffer
  - Detection Buffer
- **AlphaScreen cAMP Assay (PerkinElmer Catalog# 6760625)**
- The kit includes the following component:
  - Biotinylated cAMP
    - Anti-cAMP Acceptor Beads
    - Donor Streptavidin Beads
    - cAMP Standard
    - 10X Control Buffer
    - 1% Tween-20 Solution
- **OptiPlate (1536-well white plate) (PerkinElmer Catalog # 6005226 White or 6005226 Black)**
- Chemicals-Forskolin (Calsbiochem# 344270), Agonist (Epinephrine# Sigma E2250), Cell line -  $\beta_2$  adrenergic receptor cell line (PerkinElmer MCL-501)

\* The LANCE cAMP kit will be introduced as an "off the shelf" product in the Fall 2004.

**6 Methods**

➤ **AlphaScreen cAMP**

- 2  $\mu$ L cAMP standards, forskolin, or agonist in PBS
- 2  $\mu$ L cells diluted in Stimulation Buffer containing Acceptor Beads or Stimulation Buffer containing Acceptor Beads without cells to standards
- Incubate 30 - 60 minutes at room temperature
- 4  $\mu$ L Detection Buffer containing biotinylated-cAMP and Donor Beads
- Incubate 1 hour at room temperature
- Read on an appropriate reader



**12 Conclusion**

- Excellent performance in a 1536-well format with all three assay platforms
- Correct pharmacology as compared to literature values
- Dramatic cost savings
  - Requires less reagents/well
  - Requires less cells/well
- Ultra High Throughput Screening capability
- Simple protocols
- Easily adapted to robotic systems