Fluorescence Polarization cAMP Assay Kit

[FP]²: A Direct, Homogeneous, Cell-Based HTS Assay for cAMP

[FP]² cAMP is a fast, easy way to automate your HTS assays. Fluorescence based and proven for 96-, 384- and 1536-well miniaturization, it is a homogeneous procedure requiring no washes, no separation steps, and no radioactive disposal. [FP]² increases the performance and productivity of your cAMP HTS assays while significantly lowering reagent costs.

[FP]² Fluorescence Polarization measures the parallel and perpendicular components of fluorescence emission using plane polarized excitation. Polarization values (measured in mP units) for any fluorophore-labeled complex are inversely related to the speed of molecular rotation of that complex. During the assay, cAMP, produced by stimulating cells or membrane receptors, displaces Fluo-cAMP from the antibody. The newly unbound Fluo-cAMP rotates faster, decreasing the polarization value. The magnitude of this change in polarization is used to quantitatively measure the level of cAMP in a simple, homogeneous technique.

Fast and Optimized Protocols for Accelerated Assay Development

[FP]² Fluorescence Polarization replaces traditional multi-step, automation-resistant assay procedures, letting you screen over 100,000 cAMP assay points in one day. The entire assay, both the production of cAMP by cells and the detection of cAMP by fluorescence polarization immunoassay, is performed in the same micro-well plate by: 1) adding live cells or membranes, 2) stimulating, 3) adding Detecting Mix, 4) incubating at room temperature, and 5) reading without wash steps. Incubation time can vary from 0.5 - 24 hours, allowing flexibility.
**Expected Results**

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**[FP]² cAMP Standard Curve**

- Standard dilutions run in triplicate. The assay was incubated for 40 minutes before reading. Error bars show the mean ± 1 SD. The typical lower limit of detection for this assay is less than 0.2 pmol/well (value interpolated from standard curve for mP of zero standard - 2 SD).

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**Forskolin Dose Response**

- HEK-293 cells (13,000 per well) were stimulated with forskolin and assayed in a single plate. Error bars represent ± 1 SD of triplicate points. The displacement curve is characterized by an EC₅₀ of 14 µM (log M EC₅₀ = -4.9 ± 0.03) and a displaceable signal of 74 mP.

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**Agonist Stimulation (CRF Receptor)**

- HEK-293 cells (13,000 per well) were stimulated with forskolin and assayed in a single plate. Error bars represent ± 1 SD of triplicate points. The displacement curve is characterized by an EC₅₀ of 14 µM (log M EC₅₀ = -4.9 ± 0.03) and a displaceable signal of 74 mP.

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**Intra-Assay Precision:** Standards run on 3 days. B₀ wells contained no cAMP standard. The IC₅₀ wells contained 50 nM cAMP standard, and the NSB wells contained 2500 nM cAMP standard. The combined Z value was 0.82.

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**[FP]² cAMP Assay Kits**

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<tr>
<th>Kit</th>
<th>Code</th>
<th>Description</th>
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<td>10 plate</td>
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**How to Order**

For more information on [FP]² cAMP assay kits from PerkinElmer Life Sciences, or to place your order, call your local PerkinElmer representative. For the name and number of the one nearest you, call (800) 551-2121 in the U.S., or +32 2 717 7924 in Europe. Or visit our website at: www.perkinelmer.com/lifesciences.