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- Trockeneiszuschlag
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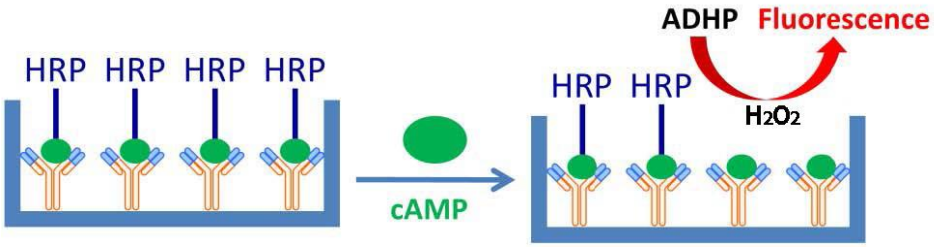
Elite™ cAMP 384-Well ELISA Assay Kit (Fluorescence)

CATALOG NUMBER: CA-C325, 1 plate (384-well)

Description

Adenosine 3', 5' cyclic monophosphate (cAMP) is an important second messenger in intracellular signal transduction. Monitoring cAMP levels is one of the most common ways to screen for agonists and antagonists of GPCRs. Elite™ cAMP ELISA Assay Kit is based on the competition between HRP-labeled cAMP and free cAMP for a fixed number of cAMP antibody binding sites. HRP-cAMP is displaced from the HRP-cAMP/anti-cAMP antibody complex by unlabeled free cAMP. In the absence of cAMP, HRP-cAMP conjugate is bound to anti-cAMP antibody exclusively. However, the unlabeled free cAMP in the test samples competes for anti-cAMP antibody with the HRP-cAMP antibody conjugate, therefore inhibits the binding of HRP-cAMP to anti-cAMP antibody.



Maximum HRP activity **Decreasing HRP activity**

Elite™ cAMP ELISA Assay Kit provides the sensitive method for detecting adenylate cyclase activity in biochemical or cell-based assay system. Compared to other ELISA cAMP assay kits, our kit eliminates the tedious acetylation step. The kit uses Elite™ Red as a fluorimetric substrate to quantify the HRP activity. The assay is specifically formulated for using 384-well microtiter-plate and easily adapted to automation. The fluorescent product formed is proportional to the activity of HRP-cAMP conjugate.

Features

- Wide reading windows
- High sensitivity
- Sensitivity (IC_{50}): 1.6 nM (1.6 pmol/ml)

Kit Components

• **Component A**: cAMP Standard (100 µM)	100 µl (store at -20 °C)
• **Component B**: Assay Buffer	5 ml
• **Component C**: HRP-cAMP Conjugate (250x)	12 µl (store at -20 °C)
• **Component D**: 10x Wash Buffer	5 ml
• **Component E**: Cell Lysis Buffer	11 ml
• **Component F**: 3% H_2O_2	50 µl
• **Component G**: Elite™ Red (100x)	100 µl (store at -20 °C)
• **Component H**: Anti-cAMP Antibody Pre-Coated 384-Well Plate	1 plate
• **Component I**: Substrate Buffer	10 ml

Storage

Keep **Component A, C, G** at -20 °C and all other Components at 4 °C; keep **Component G** in the dark.

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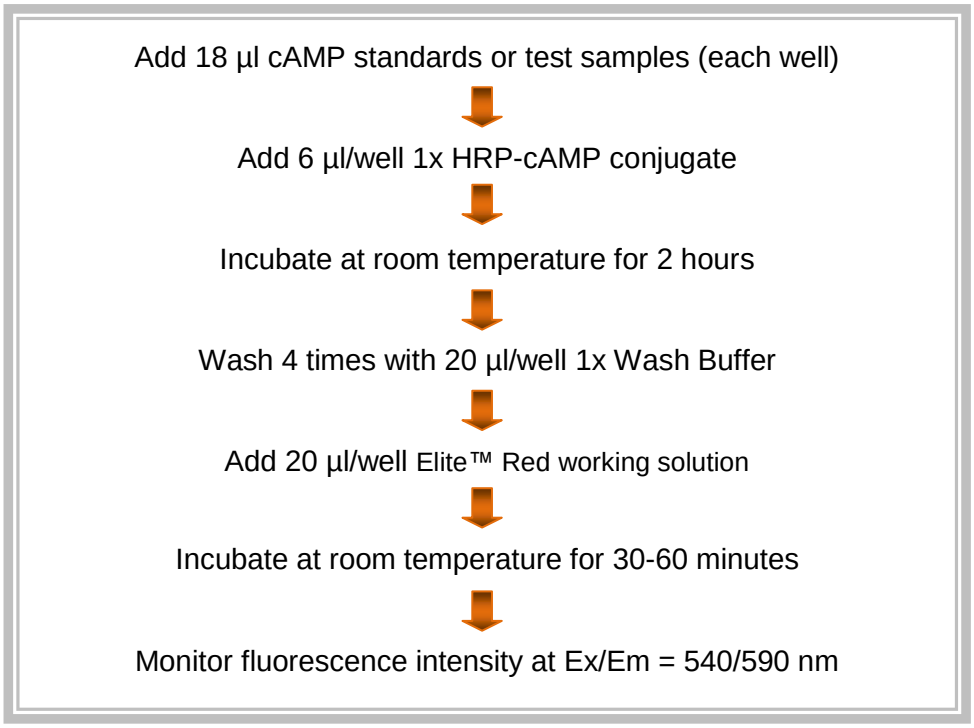
Assay Protocol (for 384-Well Plate):

Brief Summary of the Assay



1. Prepare cells or samples:

1.1 Cell Samples:

For adherent cells: Plate cells overnight in growth medium at 10,000 -14,000 cells/well for a 384-well plate.

For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellets in culture medium at 25,000-50,000 cells/well for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiment.

Treat cells as desired: The following is an example for Hela cells treated with Forskolin to induce cAMP in a 384-well plate format.

- Aspirate off cell growth medium, add 25 µl/well of 0.75 mM IBMX in Hanks and 20 mM Hepes buffer (HHBS). Incubate at room temperature for 10 minutes.
- Add 12.5 µl/well of 150 µM Forskolin in HHBS, incubate in a 5% CO2, 37 ^{o}C incubator for 15 minutes.
- Aspirate off cell solution after the incubation. And then add 25 µl/well of Cell Lysis Buffer (**Component E**), incubate at room temperature for another 10 minutes.
- This cell lysate can be assayed directly or diluted in cell lysis buffer.

Note: *Each cell line should be evaluated on an individual basis to determine the optimal cell density. Cells may be seeded the day before or on the day of the experiment depending upon the cell type and/or the effect of the test compounds.*

1.2 Tissue Samples: It is important to rapidly freeze tissues after collection (e.g., using liquid nitrogen) due to quick metabolism of cyclic nucleotides in tissue. Weigh the frozen tissue and add 10-20 µl/mg of cell lysis

buffer. Homogenize the sample on ice. Spin at top speed for 5 minutes and collect the supernatant. The supernatant may be assayed directly.

1.3 Urine, Plasma and Culture Medium Samples: Urine and plasma may be tested directly with 1:200 to 1:1000 dilutions in Lysis Buffer. Culture medium can also be tested with 1:10 to 1:200 dilutions in Lysis Buffer.

Note: *RPMI medium may contain > 350 fmol/µl cAMP.*

2. Prepare cAMP assay solutions:

2.1 Prepare serial dilution of cAMP in Assay Buffer (**Component B**) to have 1,000, 300, 100, 30, 10, 3, 1, 0.3, and 0 nM cAMP diluted solutions. Store on ice or 4 ^{o}C.

Note: *The unused 100 µM cAMP stock solution should be aliquoted and stored at -20 ^{o}C.*

2.2 Prepare 1X HRP-cAMP conjugate working solution by adding 12 µl 250x HRP-cAMP Conjugate (**Component C**) into 3 ml of Assay Buffer (**Component B**). Store on ice or 4 ^{o}C.

2.3 Prepare 1X washing buffer by adding 1 ml of 10X Wash Buffer (**Component D**) to 9 ml distilled water.

3. Run cAMP assay:

3.1. All the assay wells will be prepared in the following orders: A) cAMP standards, control, or tests samples; B) HRP-cAMP conjugate.

3.2. Add 18 µl/well of the cAMP diluted solution (from Step 2.1) and test samples into each well of the anti-cAMP Ab pre-coated 384-well plate (**Component H**). It is recommended to duplicate the assays for each standard and test sample. Incubate at room temperature for 5 to 10 minutes.

3.3. Add 6 µl/well of 1X HRP-cAMP conjugate working solution (from Step 2.2). Incubate at room temperature for 2 hours by placing the plate on shaker.

3.4. Aspirate plate contents, and wash 4 times with 20 µl/well of 1X wash buffer (from Step 2.3).

3.5. Prepare Elite™ Red working solution by adding 100 µl of 100 X Elite™ Red stock solution (**Component G**) and 11.5 µl of 3% H_2O_2 (**Component F**) into 10 ml of Substrate Buffer (**Component I**).

Note: *The Elite™ Red working solution is not stable, use it promptly.*

3.6. Add 20 µl/well of Elite™ Red working solution (from Step 3.5) into each well, and incubate at room temperature for 30 minutes to 1 hour, protected from light.

3.7. Monitor the fluorescence increase at Ex/Em = 540/590 nm (cutoff 570 nm) by using a fluorescence plate reader (top read mode). For best result, get the reading within 1-2 hours after incubation.

Data Analysis:

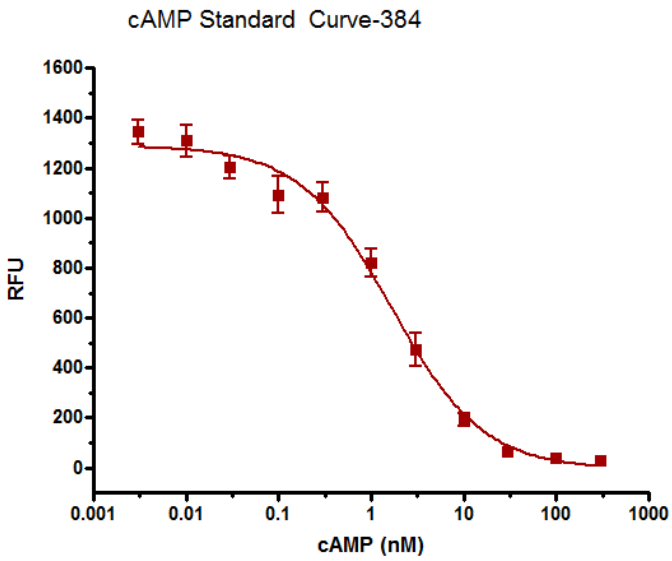


Figure 1. cAMP dose response was measured with Elite™ cAMP ELISA Assay Kit in a solid black 384-well plate with a Gemini microplate reader. The kit can detect as low as 0.1 nM cAMP in a 24 µl reaction.