# Screen Quest™ TR-FRET No Wash cAMP Assay Kit

Catalog number: 36379, 36380, 36381
Unit size: 1 plate, 10 plates, 50 plates

| Component | Storage | Amount (Cat No. 36379) | Amount (Cat No. 36380) | Amount (Cat No. 36381) |
|---|---|---|---|---|
| Component A: Anti cAMP-trFluor™ Eu | Refrigerated (2-8 °C), Minimize light exposure | 1 vial | 1 vial | 5 vials |
| Component B: cAMP-trFluor™ 650 | Refrigerated (2-8 °C), Minimize light exposure | 1 vial | 1 vial | 5 vials |
| Component C: cAMP Standard | Refrigerated (2-8 °C), Minimize light exposure | 1 vial (33 µg) | 1 vial (33 µg) | 1 vial (33 µg) |
| Component D: Cell Lysis Buffer | Refrigerated (2-8 °C), Minimize light exposure | 1 bottle (10 mL) | 1 bottle (100 mL) | 5 bottles (100 mL/bottle) |
| Component E: Diluent | Refrigerated (2-8 °C), Minimize light exposure | 1 bottle (10 mL) | 1 bottle (100 mL) | 5 bottles (100 mL/bottle) |

## OVERVIEW

Screen Quest™ TR-FRET No Wash cAMP Assay Kit provides a convenient assay method for monitoring the activation of adenylyl cyclase in G-protein coupled receptor systems. Compared to other commercial ELISA cAMP assay kits, this homogenous cAMP assay kit does not require a wash step or the acetylation step. The assay is based on the competition for a fixed number of anti-cAMP antibody binding sites between the trFluor™ 650 labeled cAMP tracer and non-labeled free cAMP. The anti-cAMP antibody is labeled with trFluor™ Eu while the cAMP tracer is labeled with trFluor™ 650. In the absence of cAMP, trFluor™ 650-cAMP conjugate is bound to trFluor™ Eu-labeled anti-cAMP antibody exclusively to have a strong FRET signal. While the unlabeled free cAMP is present in the test sample, it competes for the trFluor™ Eu-labeled anti-cAMP antibody conjugate binding sites, therefore inhibits the binding of trFluor™ 650-cAMP to anti-cAMP antibody. The trFluor™ 650 labeled cAMP tracer only has fluorescence lifetime of nanosecond while TR Fluor™ Eu-labeled anti-cAMP antibody-bound fluorescent cAMP tracer has much longer fluorescence lifetime of lanthanide fluorophore. The magnitude of time -resolved fluorescence signal (TR- FRET) signal is proportional to the concentration of cAMP in a sample. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format, and is convenient for monitoring the cAMP activity with ultra-specificity and sensitivity in G-protein coupled receptor systems.

## KEY PARAMETERS

**Fluorescence microplate reader**

| | |
|---|---|
| Recommended plate | Solid black and/or Black wall/clear bottom |
| Instrument specification(s) | Time-resolved |

## CELL PREPARATION

**For adherent cells**

Plate cells overnight in growth medium at 30,000 -100,000 cells/well for a 96-well plate.

**For non-adherent cells**

Centrifuge the cells from the culture medium and then suspend the cell pellets in culture medium at 100,000-300,000 cells/well for a 96-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 96-well plate format. 25µL cells in growth medium, add 25 µL/well 100 µM Forskolin in Hanks and 20 mM Hepes buffer (HHBS), incubate in a 5% CO2 , 37 °C incubator for 15 minutes.

**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.

## PREPARATION OF STOCK SOLUTIONS

*Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles*

**cAMP standard (1mM)**

Add 100 µL Diluent (Component E) to cAMP Standard (Component C) and mix them well.

**Note:** The unused cAMP standard can be aliquoted and stored at -20 °C.

## PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/36379

**cAMP standard**
1 mM stock soluton can be diluted to 11200 nM followed by 4X dilutions.

## PREPARATION OF WORKING SOLUTION

**Anti cAMP-trFluor™ Eu working solution**

Add 50 µL of solution (Component A) to 2.5 mL of Cell Lysis Buffer (Component D).

**Note:** Make soultion just before use and as per needed.

**cAMP-trFluor™ 650 working solution**

Add 50 µL of solution (Component B) to 2.5 mL of Cell Lysis Buffer (Component D).

**Note:** Make soultion just before use and as per needed.

## SAMPLE EXPERIMENTAL PROTOCOL

**Table 1.** Layout of cAMP standards and test samples in a solid black 96-well microplate. CS = cAMP standard (CS1-CS7); BL = blank control;

TS = test sample.

| BL | BL | TS | TS |
|---|---|---|---|
| CS1 | CS1 | ... | ... |
| CS2 | CS2 | ... | ... |
| CS3 | CS3 | | |
| CS4 | CS4 | | |
| CS5 | CS5 | | |
| CS6 | CS6 | | |
| CS7 | CS7 | | |

**Table 2.** Reagent composition for each well.

| Well | Volume | Reagent |
|---|---|---|
| CS1-CS7 | 25 µL | Serial Dilution |
| BL | 25 µL | Diluent (Component E) |
| TS | 25 µL | Test Sample |

**Table 3.** Overview of the protocol

| cAMP Standard | | | Cells | | |
|---|---|---|---|---|---|
| Negative Control | Positive Control | Standard Curve | Negative Control | Non-stimulated | Stimul |
| 25 µL Diluent | 25 µL Diluent | 25 µL Standard | 25 µL cells | 25 µL cells | 25 µL |
| 25 µL Compound Buffer | 25 µL Compound Buffer | 25 µL Compound Buffer | 25 µL Compound Buffer | 25 µL Compound Buffer | 25 µ Compo |
| Incubate 30 min at RT | | | | | |
| 25 µL Lysis Buffer | 25 µL cAMP-trFluor™ 650 working solution | 25 µL cAMP-trFluor™ 650 working solution | 25 µL Lysis Buffer | 25 µL cAMP-trFluor™ 650 working solution | 25 µ cAM trFluc 650 worki soluti |
| 25 µL Anti cAMP-trFluor™ Eu working solution | | | | | |
| Incubate 30min at RT | | | | | |

**Table 4.** Compatible HTRF® plate readers

| Manufacturers | Instruments |
|---|---|
| Berthhold Technologies | Tristar$^2$ S; Mithras LB 940; Mithras$^2$ LB 943 |
| Hidex | Sense; Sense Beta Plus |
| Molecular Devices | Spectra Max i3X; Spectramax Paradigm; Spectramax M5e; Spectramax 3 |
| Thermo Scientific | Varioskan Lux |
| Biotek | Synergy Neo2; Cytation 5; Cytation 3; Synergy H1; Synergy 2 |
| BMG Labtech | PHERAstar; CLARIOstar; POLARstar Omega; Fluostar Omega |
| Tecan | Spark 10M; Infinite M100 Pro; Infinite F500; Infinite F200 Pro |

**cAMP assay in cell lysate**

1. Prepare and add cAMP standards (CS), blank controls (BL) and test samples (TS) according to the layout provided in Table 1 and Table 2. For a 384-well plate, use 12.5 µL of each corresponding reagent instead of 25 µL.

   **Note:** Test samples could be Non-stimulated and/or stimulated samples.

2. Add 25 µL of treatment (Buffer or Compound resuspended in buffer- See table 3) into each well of cAMP standard, blank control, and test samples to make the total cAMP assay volume of 50 µL/well. For a 384-well plate, add 12.5 µL of working solution into each well for a total volume of 25 µL/well.
3. Incubate the reaction at room temperature for 30 minutes.
4. Add 25 µL of cAMP-trFluor™ 650 working solution into each well of cAMP standard, blank control, and test samples to make the total cAMP assay volume of 75 µL/well. For a 384-well plate, add 12.5 µL of working solution into each well for a total volume of 37.5 µL/well.

   **Note:** For negative controls, Lysis Buffer can be added.

5. Add 25 µL of cAMP-trFluor™ Eu working solution into each well of cAMP standard, blank control, and test samples to make the total cAMP assay volume of 100 µL/well. For a 384-well plate, add 12.5 µL of working solution into each well for a total volume of 50 µL.
6. Incubate the reaction at room temperature for 30 minutes.
7. Read on a compatible TR-FRET reader.

## EXAMPLE DATA ANALYSIS AND FIGURES

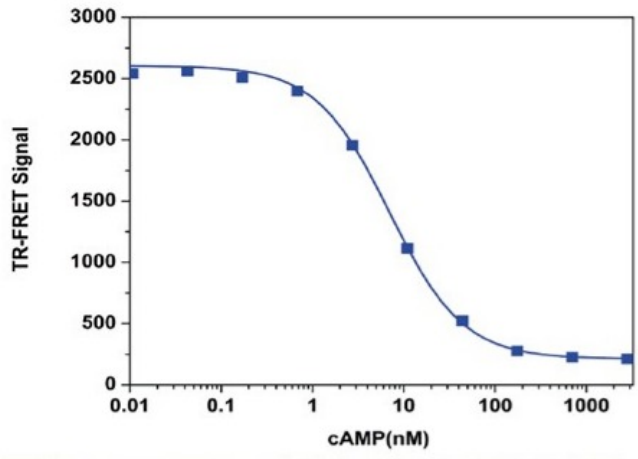


**Figure 1.** cAMP does response was measured with Screen Quest™ TR-FRET No Wash cAMP Assay Kit (Cat #36379) and fluorescence was measured on CLARIOstar microplate reader (BMG Labtech). The assay demonstrated a detection sensitivity as low as 1 nM cAMP.

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