Screen Quest™ 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	Cat No. 36380	Cat No. 36381
Component A: Anti cAMP-trFluor™ Eu	Refrigerate (2-8 °C), Minimize light exposure	1 vial	1 vial	5 vials
Component B: cAMP-trFluor™ 650	Refrigerate (2-8 °C), Minimize light exposure	1 vial	1 vial	5 vials
Component C: cAMP Standard	Refrigerate (2-8 °C), Minimize light exposure	1 vial (33 µg)	1 vial (33 µg)	1 vial (33 µg)
Component D: Cell Lysis Buffer	Freeze (<-15 °C), Minimize light exposure	10 mL	100 mL	5 bottles (100 mL/bottle)
Component E: Diluent	Refrigerate (2-8 °C), Minimize light exposure	10 mL	100 mL	5 bottles (100 mL/bottle)

OVERVIEW

Screen Quest™ 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 fluorescent cAMP tracer and non-labeled free cAMP. Free cAMP displaces the fluorescent cAMP tracer from the HRP-cAMP/anti-cAMP antibody complex. The anti-cAMP antibody is labeled with our TR Fluor™ Eu while the cAMP tracer contains our trFluor™ 650. In the absence of cAMP, trFluor™ 650-cAMP conjugate is bound to TR Fluor™ Eu-labeled anti-cAMP antibody exclusively to have a strong FRET. However, the unlabeled free cAMP in the test sample competes for the TR Fluor™ Eu-labeled anti-cAMP antibody conjugate, 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 value due to the TR-FRET. The magnitude of FRET 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.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Instrument specification(s):	Time-resolved
Recommended plate:	Solid black and/or Black wall/clear bottom

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 SOLUTION

cAMP standard

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

PREPARATION OF WORKING SOLUTION

1. Anti cAMP-trFluor™ Eu working solution:
Add 55 µL (**Cat. # 36379**) or 550 µL (**Cat. # 36380 or # 36381**) of Diluent (Component E) into the vial of Anti cAMP-trFluor™ Eu (Component A). Add 50 µL of reconstituted solution to 2.5 mL of Cell Lysis Buffer (Component D).

Note Make soultion just before use and as per needed.

2. cAMP-trFluor™ 650 working solution:
Add 55 µL (**Cat. # 36379**) or 550 µL (**Cat. # 36380 or # 36381**) of Diluent (Component E) into the vial of cAMP-trFluor™ 650 (Component B). Add 50 µL of reconstituted solution to 2.5 mL of Cell Lysis Buffer (Component D).

Note Make soultion just before use and as per needed.

SAMPLE EXPERIMENTAL PROTOCOL

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% CO_2, 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.

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		

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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

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 (Compound resuspended in buffer) 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.

Overview of the protocol:

cAMP Standard			Cells		
Negative Control	Positive Control	Standard Curve	Negative Control	Non-stimulated	Stimulated
25 µL Diluent	25 µL Diluent	25 µL Standard	25 µL cells	25 µL cells	25 µL cells
25 µL Compound Buffer	25 µL Compound Buffer	25 µL Compound Buffer	25 µL Compound Buffer	25 µL Compound Buffer	25 µL Compound
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 µL cAMP-trFluor™ 650 working solution
25 µL Anti cAMP-trFluor™ Eu working solution					
Incubate 30min at RT					

Table 3. 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

EXAMPLE DATA ANALYSIS AND FIGURES

Results are Relative Fluorescence Units at 665nm and 620nm. Ratio is calculated as the F_{665nm} / F_{620nm} ratio and expressed in Δ F%.

$R = F_{665nm}/F_{620nm}$

$\Delta F\% = 100\% \times (R_{sample}-R_{neg})/R_{neg}$

Draw a standard curve by plotting Δ F% versus cAMP concentration as shown in the graph below.

The reading (Delta F) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate cAMP samples. We recommend using the Online Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

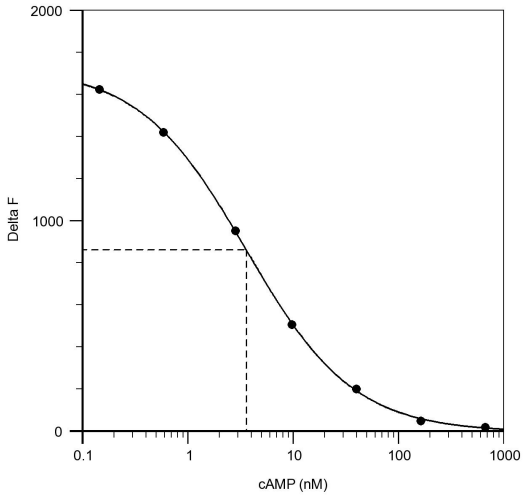

Figure 1. cAMP dose response was measured with Screen Quest™ FRET No Wash cAMP Assay Kit using a ClarioStar microplate reader (BMG).

DISCLAIMER