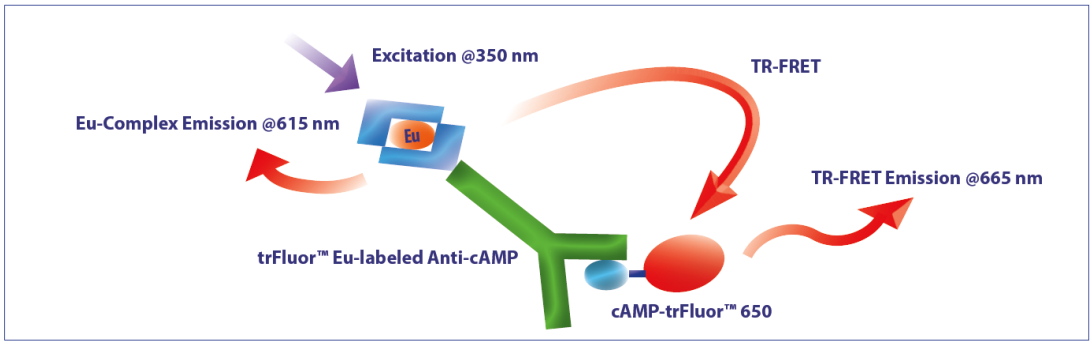
Screen Quest™ TR-FRET No Wash cAMP Assay Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 36379 (1 plate), 36380 (10 plates), 36381 (50 plates)	Keep in -20°C Avoid exposure to light	Time-resolved fluorometric microplate reader

Introduction

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. Screen Quest™ No Wash FRET cAMP Assay Kit provides a convenient assay method for monitoring the activation of adenylyl cyclase in G-protein coupled receptor systems. Compared to ELISA cAMP assay kits, this homogenous cAMP assay kit does not require a wash step or the acetylation step. In this assay, the Anti-cAMP antibody is labeled with our trFluor™ Eu while the cAMP tracer is labeled with trFluor™ 650. In the absence of cAMP, cAMP- trFluor™ 650 conjugate is bound to trFluor™ Eu-labeled Anti-cAMP antibody exclusively to have a strong TR-FRET Emission at 655nm. However, free cAMP in the test sample competes for the trFluor™ Eu-labeled anti-cAMP antibody conjugate, therefore inhibits the binding of cAMP-trFluor™ 650 to trFluor™ Eu-labeled anti-cAMP antibody. The trFluor™ 650 labeled cAMP tracer only has fluorescence lifetime of nanosecond while trFluor™ Eu-labeled anti-cAMP antibody-bound fluorescent cAMP tracer has much longer fluorescence lifetime value due to the TR-FRET.



The magnitude of TR-FRET is proportional to the concentration of cAMP in a sample. Screen Quest™ No Wash TR- FRET cAMP 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.

Kit Components

Components	Amount		
	Cat. # 36379 (1 plate)	**Cat. # 36380 (10 plates)**	**Cat. # 36381 (50 plates)**
Component A: Anti cAMP-trFluor™ Eu	1 vial	1 vial	5 vials
Component B: cAMP-trFluor™ 650	1 vial	1 vial	5 vials
Component C: cAMP Standard	1 vial (33 µg)	1 vial (33 µg)	1 vial (33 µg)
Component D: Cell Lysis Buffer	10 mL	100 mL	5 bottles (100 mL/bottle)
Component E: Diluent	10 mL	100 mL	5 bottles (100 mL/bottle)

Assay Protocol for One 96-well Plate

1. **Prepare samples:**

   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.

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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 ^oC 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.

2. **Prepare cAMP Standard:** add 100 µL Component E (Diluent) to Component C (cAMP Standard) to make 1mM stock solution. Dilution can be carried out with the diluent (or with cell culture media). Add 2.24 µL 1mM stock solution into 200µL Diluent to make 11200nM standard, and then get 50µL 11200nM cAMP standard into 150µL Dilent to get 2800nM Standard, and repeat this procedure to make the serial dilutions. The cAMP standard dilutions are: 11200, 2800, 700, 175, 43.75, 10.94, 2.73, 0.68 nM (final concentration of 2800~ 0.17 nM cAMP per well).

3. **Prepare Anti cAMP-trFluor™ Eu working solution**:
Add 55µL Component E into vial A to reconstitute Anti cAMP-trFluor™ Eu for #36379, (or 550µL Component E into vial A for #36380 and #36381), and then prepare working solution by adding 50µL reconstituted Component A to 2.5mL Cell Lysis Buffer (Component D), or make the needed volume proportionally. Prepare before use!

4. **Prepare cAMP-trFluor™ 650 working solution:**
Add 55µL Component E into vial B to reconstitute cAMP-trFluor™ 650 for #36379, (or 550µL Component E into vial A for #36380 and #36381), and then prepare working solution by adding 50µL reconstituted Component B to 2.5mL Cell Lysis Buffer (Component D), or make the needed volume proportionally. Prepare before use!

5. **Run cAMP assay:**

Run cAMP Assay (including the standard curve and cell based assay) as instruction in the following table:

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

Read on a compatible TR-FRET reader.

Data Analysis

Results are Relative Fluorescence Units at 665nm and 620nm. Ratio is calculated as the F_{665nm} / F_{620nm} ratio and expressed in Delta F%.
$R= F_{665nm}/F_{620nm}$
Delta F%= 100% x $(R_{sample}-R_{neg})/R_{neg}$
Draw a standard curve by plotting Delta F% versus cAMP concentration as shown in the graph below.

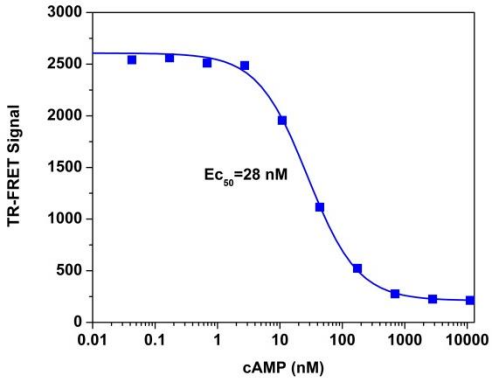

Figure 1. cAMP dose response was measured with Screen Quest™ cAMP FRET Assay Kit using a ClarioStar microplate reader (BMG). The kit can detect as low as 1 nM cAMP .

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

References

1. Alonso GD, Schoijet AC, Torres HN, Flawia MM. (2006) TcPDE4, a novel membrane-associated cAMP-specific phosphodiesterase from Trypanosoma cruzi. Mol Biochem Parasitol, 145, 40.
2. Bader S, Kortholt A, Snippe H, Van Haastert PJ. (2006) DdPDE4, a novel cAMP-specific phosphodiesterase at the surface of dictyostelium cells. J Biol Chem, 281, 20018.
3. Charlie NK, Thomure AM, Schade MA, Miller KG. (2006) The Dunce cAMP phosphodiesterase PDE-4 negatively regulates G alpha(s)-dependent and G alpha(s)-independent cAMP pools in the Caenorhabditis elegans synaptic signaling network. Genetics, 173, 111.
4. Zhang, J. H., Chung, D. Y., Oldenburg, K. R. (1999) A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J. Biomol. Screening*, 4: 67-73.

Warning: This kit is only sold to our authorized distributors and end users. It is covered by a pending patent. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest®. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.