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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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cAMP TR-FRET Assay Kit

Powered by Bioauxilium's THUNDER™ TR-FRET Technology

Item No. 500293

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item Name	960 wells Quantity/Size	4,800 wells Quantity/Size	19,200 wells Quantity/ Size
400456	cAMP Standard	1 vial/100 µl	1 vial/100 µl	1 vial/100 µl
400457	Biotin-cAMP (200X)	1 vial/25 µl	1 vial/125 µl	1 vial/500 µl
400458	Europium-Labeled Streptavidin (200X)	1 vial/25 µl	1 vial/125 µl	1 vial/500 µl
400459	Acceptor-Labeled cAMP Antibody (100X)	1 vial/50 µl	1 vial/250 µl	1 vial/1 ml
400460	cAMP Detection Buffer (5X)	2 vials/1 ml	1 bottle/10 ml	1 bottle/40 ml

To request this kit in bulk quantities, please contact our Sales department. If any of the items listed above are damaged or missing, please contact our Customer Service department. Sales and Customer Service departments can be reached at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

Do not mix or substitute reagents or materials from other kit lots or kits. Kits are quality control tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

We cannot guarantee the performance of the product outside the conditions detailed in this kit booklet.

Before You Start

Please note the following:

ONLY white plates should be used for TR-FRET.

DO NOT modify the assay protocol or volumes.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed at -80°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader equipped with a TR-FRET option
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. Low-volume 384-well, or half-area 96-well, white plates
5. Stimulation buffer
6. Hank's balanced salt solution (HBSS) (1X), available from Gibco™ (Catalog No. 14025-092)
7. Versene™ (1X), available from Gibco™ (Catalog No. 15040-066)
8. HEPES Buffer Solution (1M), available from Gibco™ (Catalog No. 15630-080)
9. BSA
10. Forskolin, available from Cayman Chemical (Item No. 11018)
11. IBMX, available from Cayman Chemical (Item No. 13347)
12. DMSO
13. Adhesive sealing film for plates

INTRODUCTION

Background

Adenosine 3',5' cyclic monophosphate (cAMP) is a ubiquitous cellular second messenger and critical component of G protein-coupled receptor (GPCR) pathways that use $G\alpha_s$ or $G\alpha_i$ subunits for signaling.[1] cAMP is synthesized by membrane-bound adenylate cyclase from ATP after the binding of ligands to $G\alpha_s$-linked GPCRs. It activates various targets, including PKA, the guanine nucleotide exchange factor EPAC, and cyclic nucleotide-gated ion channels, that have roles in a wide variety of cellular processes from gene transcription to calcium signaling.[1,2] cAMP synthesis is prevented by $G\alpha_i$-linked GPCR activation, which inhibits adenylate cyclase, and its activity can be attenuated by phosphodiesterase-mediated hydrolysis to AMP.[1] Therefore, the concentration of cAMP in a cell is a function of the ratio of synthesis from ATP by adenylate cyclase and its rate of breakdown to AMP by phosphodiesterases.

About This Assay

This cAMP TR-FRET Assay Kit uses a homogeneous TR-FRET competitive immunoassay method amenable to rapid measurement of GPCR agonist- or antagonist-induced cAMP production in cells. The amount of reagents provided is sufficient for testing either 960, 4,800, or 19,200 wells, depending on the size of the kit.

Principle Of This Assay

This assay is a homogenous TR-FRET-based competitive immunoassay designed to measure GPCR agonist- or antagonist-induced cAMP production in cells. (Figures 1, below and 2, on page 9). GPCRs that couple with adenylate cyclase will increase or decrease intracellular cAMP levels, which will be detected with a fluorophore-labeled antibody reactive to cAMP, a biotin-cAMP conjugate, and fluorophore-labeled streptavidin.

Figure 1. Schematic of the TR-FRET cAMP assay principle in the absence of free cAMP

Figure 2. Schematic of the TR-FRET cAMP assay principle in the presence of free cAMP

Cells are first stimulated to either increase or decrease the cAMP levels. cAMP is detected during the lysis step in a simple “add-incubate-measure” format. The assay is based on the competition between free cAMP and a europium-labeled cAMP tracer complex for binding to a cAMP-specific monoclonal antibody labeled with a far-red acceptor fluorophore (Acceptor-Labeled cAMP Antibody). The tracer complex is formed by the tight interaction between biotinylated cAMP (Biotin-cAMP) and streptavidin labeled with a europium chelate donor (Europium-Labeled Streptavidin). In the absence of free cAMP, the Acceptor-Labeled cAMP Antibody is bound to the Europium-Labeled Streptavidin/Biotin-cAMP tracer complex, which brings the two dyes into close proximity. Excitation of the donor Eu chelate molecules with a flash lamp (320 or 340 nm) or laser (337 nm) triggers a FRET from the donor to the acceptor molecules, which, in turn, emit a TR-FRET signal at 665 nm. Residual energy from standard or cell lysate test samples competes with the Europium-Labeled Streptavidin/Biotin-cAMP tracer complex for binding to the Acceptor-Labeled cAMP Antibody, causing a decrease in the TR-FRET signal. As a result, the specific signal at 665 nm will be inversely proportional to the cAMP concentration in the sample. Data can be expressed as either the signal at 665 nm or the 665/615 nm ratio.

PRE-ASSAY PREPARATION

Assay Optimization

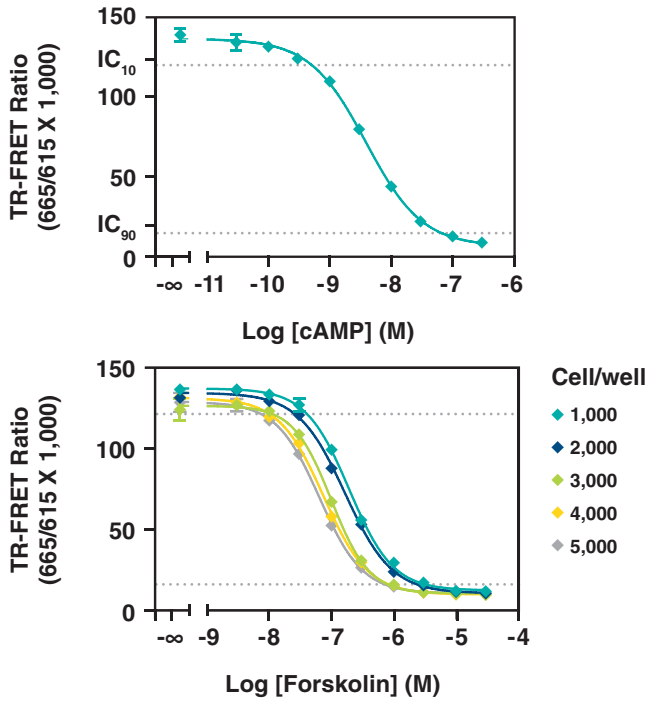
The THUNDER™ TR-FRET cAMP Assay should be optimized following the assay development workflow described in Table 1, on page 11.

Step	Purpose
1	Select a suitable cell line or primary cells.
2	Determine the sensitivity (IC_{50} value) and working range (IC_{10}-IC_{90}) of the cAMP assay by running a standard curve.
3	Determine the optimal cell density giving the highest signal-to-background (S/B) ratio while staying within the assay working range (IC_{10}-IC_{90}). a. **G_s Assay:** Run a concentration-response experiment using a full agonist or forskolin at different cell densities. b. **G_i Assay:** Run a concentration-response experiment using forskolin at different cell densities. Convert signals to cAMP levels to determine the EC_{50} value of forskolin to be used for the agonist assay.
4	Determine agonist potency (EC_{50} value) by running an agonist concentration-response curve. a. **G_i Assay:** Use the EC_{50} forskolin concentration (based on cAMP levels).
5	Determine antagonist potency (IC_{50} value) by running an antagonist concentration-response curve. a. **G_s Assay:** Use the EC_{50} agonist concentration (based on cAMP levels). b. **G_i Assay:** Use the EC_{50} forskolin and the EC_{50} agonist concentration (based on cAMP levels).

Table 1. Assay workflow

Assay Development Guidelines

1. **IBMX Concentration:** IBMX is a widely used non-selective inhibitor of cAMP phosphodiesterases. It is recommended to use IBMX at a concentration of 0.5 mM in the stimulation buffer. IBMX does not reduce the signal in cAMP standard curves at this concentration. The effect of IBMX on compound potency and the optimal concentration should be determined according to the assays and cellular models being used. IBMX can be prepared at a high concentration (500 mM), aliquoted, and stored at -20°C. Avoid repeated freeze/thaw cycles.
2. **cAMP Standard Curve:** A cAMP standard curve should be included with each run both to verify that the assay generates the expected IC_{50} value and S/B ratio and to convert the TR-FRET signals into cAMP levels. It is essential that the assay conditions, such as cell density, forskolin concentration, and agonist concentration, are optimized so the measured TR-FRET signals fall within the working range of the standard curve (defined as cAMP concentrations between IC_{10}-IC_{90}). Signals at low cAMP concentrations are very sensitive to variations in cAMP concentrations, whereas signals at high cAMP concentrations are less sensitive to variations in cAMP concentrations.
3. **Cell Number:** It is recommended to generate either forskolin (G_s- and G_i-coupled receptors) or full agonist (G_s receptors) concentration-response curves at different cell densities in order to determine the optimal cell number per well. Testing 1,000 to 10,000 cells per well is recommended. The optimal cell number will be the one for which the forskolin or agonist concentration-response curve covers most of the working range of the cAMP standard curve, while maximizing the assay S/B ratio. In Figure 3, on page 13, the optimal cell concentration selected for subsequent experiments would be 4,000 cells/well.



Cells/well	1,000	2,000	3,000	4,000	5,000
S/B	12	12	13	14	12
EC_{50} Forskolin (nM)	192	164	99	75	64

Figure 3. Determination of optimal cell density Top Panel: cAMP standard curve **Bottom Panel:** Cell and forskolin cross-titration. The forskolin concentration-response curve shows 4,000 cells/well provides a response that falls within the working range of the cAMP curve while maximizing the assay S/B ratio.

4. **Forskolin Concentration:** An optimized forskolin concentration is required to produce G_i agonist concentration-response curves. Using forskolin at its EC_{50} concentration based on cAMP levels is recommended. The value should correspond to the forskolin EC_{80}-EC_{90} value based on the TR-FRET signal. *NOTE: The forskolin concentration should not exceed the working range (IC_{10}) of the cAMP standard curve.*
5. **Agonist Concentration:** The presence of an agonist is required to produce G_s and G_i antagonist concentration-response curves. Using the agonist at its EC_{50} concentration based on cAMP levels is recommended. The value should correspond to the agonist EC_{80}-EC_{90} value based on the TR-FRET signal. *NOTE: The agonist concentration should not exceed the working range (IC_{10} for a G_s-coupled receptor; IC_{90} for a G_i-coupled receptor) of the cAMP standard curve.*
6. **Stimulation Time and Temperature:** Stimulation should be completed for 30 minutes at room temperature. Optimize by evaluating stimulation responses from 15 to 60 minutes at either room temperature or 37°C.

Reagent Preparation

The instructions described below are for testing the entire number of wells in each kit. Adjust volumes accordingly when testing fewer wells. Prepare only as much reagent as is needed on the day of the assay.

Bring all reagents to room temperature prior to use.

Centrifuge all tubes before use to improve recovery of content (2,000 x g, 10-15 seconds).

Use ultrapure water (18 MΩ·cm) to dilute the cAMP Detection Buffer (5X).

NOTE: It is recommended to test all standards in triplicate and samples at least in duplicate.

1. **Stimulation Buffer**

   The recommended stimulation buffer contains HBSS (1X), 5 mM HEPES, 0.5 mM IBMX, and 0.1% BSA (pH 7.4).

   To prepare 15 ml of stimulation buffer add the following to one tube:

   14 ml of HBSS (1X)

   75 µl of 1M HEPES

   15 µl of 500 mM IBMX dissolved in DMSO

   200 µl of 7.5% BSA

   Adjust pH to 7.4 with 0.1 N NaOH and dilute to a final volume of 15 ml with HBSS (1X).

NOTE: Addition of BSA may not be essential. Alternative buffers, such as cell culture medium containing 10% FBS and phenol red, can also be used. The IBMX concentration may need further optimization when working with different cell lines.

2. **cAMP Standard Serial Dilutions**

   Prepare standard solutions just before use and use within one hour. Do NOT store the standard solutions.

   To prepare the standard solutions for use: obtain 10 clean test tubes and label them #1-10. Alternatively, serial dilutions can be conducted using a 96-well polypropylene plate.

   Prepare serial dilutions for the standard curve as indicated in Table 2, on page 16, using the cAMP Standard (Item No. 400456) and stimulation buffer. Change the pipette tip between each standard dilution. Mix each tube thoroughly between each transfer.

   Each standard well requires 5 µl of serially diluted standards at a concentration 4X the concentration necessary in the assay. This cAMP Standard (4X) solution will be diluted to 1X when added to the other reagents.

Tube	Volume of cAMP Standard	Volume of Stimulation Buffer (µl)	cAMP Standard (4X) (M)	cAMP in Final Standard Curve (M)
1	3 µl of 50 µM cAMP	122	1.2E-06	3.0E-7
2	30 µl of tube 1	60	4.0E-07	1.0E-7
3	30 µl of tube 2	70	1.2E-07	3.0E-8
4	30 µl of tube 3	60	4.0E-08	1.0E-8
5	30 µl of tube 4	70	1.2E-08	3.0E-9
6	30 µl of tube 5	60	4.0E-09	1.0E-9
7	30 µl of tube 6	70	1.2E-09	3.0E-10
8	30 µl of tube 7	60	4.0E-10	1.0E-10
9	30 µl of tube 8	70	1.2E-10	3.0E-11
10	0	70	0	0

Table 2. Standard curve serial dilutions

3. **cAMP Detection Buffer (1X)**

   Mix the cAMP Detection Buffer (5X) (Item No. 400460) end-over-end before use. The thawed cAMP Detection Buffer (5X) will be stable for 1 month when stored at 4°C or for longer periods of time when stored at -80°C.

   Add 1 ml (960 wells), 10 ml (4,800 wells), or 40 ml (19,200 wells) of cAMP Detection Buffer (5X) to 4 ml (960 wells), 40 ml (4,800 wells), or 160 ml (19,200 wells) of ultrapure water. Store unused cAMP Detection Buffer (1X) at 4°C; it will be stable for approximately two weeks.

   NOTE: The cAMP Detection Buffer (1X) is designed to lyse the cells and must be used only for the preparation of Antibody Working Solution (1X) and Europium-Labeled Streptavidin/Biotin-cAMP Tracer Mix (1X).

4. **Antibody Working Solution (1X)**

   NOTE: Each well requires 5 µl of Antibody Working Solution (1X).

   Prepare and mix just before use.

   Add 50 µl (960 wells), 250 µl (4,800 wells), or 1,000 µl (19,200 wells) of Acceptor-Labeled cAMP Antibody (Item No. 400459) to 4.95 ml (960 wells), 24.75 ml (4,800 wells), or 99 ml (19,200 wells) cAMP Detection Buffer (1X) and mix by inverting gently. *NOTE: Do not vortex.*

   Store thawed Acceptor-Labeled cAMP Antibody (100X) at 4°C; it will be stable for approximately two days. If all unused Acceptor-Labeled cAMP Antibody (100X) will not be used within two days, store it at -80°C.

   Store unused Antibody Working Solution (1X) at 4°C; it will be stable for approximately one day. If all unused Antibody Working Solution (1X) will not be used within one day, aliquot and store at -80°C. Avoid repeated freeze/thaw cycles.

5. **Europium-Labeled Streptavidin/Biotin-cAMP Tracer Mix (1X)**

   NOTE: Each well requires 5 µl of Europium-Labeled Streptavidin/Biotin-cAMP Tracer Mix (1X).

   Prepare and mix just before use.

   Co-dilute Europium-Labeled Streptavidin (Eu-SA) (200X) (Item No. 400458) and Biotin-cAMP (200X) (Item No. 400457) with cAMP Detection Buffer (1X) as described below.

   5 ml of Eu-SA/Biotin-cAMP Tracer Mix (1X) (960 wells): Add 4,950 µl of cAMP Detection Buffer (1X) to a tube. Add 25 µl of Eu-SA (200X) and mix gently. Add 25 µl of Biotin-cAMP (200X) and mix gently. Incubate for at least 15 minutes at room temperature.

   25 ml of Eu-SA/Biotin-cAMP Tracer Mix (1X) (4,800 wells): Add 24.75 ml of cAMP Detection Buffer (1X) to a tube. Add 125 µl of Eu-SA (200X) and mix gently. Add 125 µl of Biotin-cAMP (200X) and mix gently. Incubate for at least 15 minutes at room temperature.

   100 ml of Eu-SA/Biotin-cAMP Tracer Mix (1X) (19,200 wells): Add 99 ml of cAMP Detection Buffer (1X) to a tube. Add 500 µl of Eu-SA (200X) and mix gently. Add 500 µl of Biotin-cAMP (200X) and mix gently. Incubate for at least 15 minutes at room temperature.

   Store unused Eu-SA (200X) and unused Biotin-cAMP (200X) at 4°C; they will be stable for approximately two days. If all unused Eu-SA (200X) and unused Biotin-cAMP (200X) will not be used within two days, aliquot and store at -80°C. Avoid repeated freeze/thaw cycles. The unused Eu-SA/Biotin-cAMP Tracer Mix (1X) can be stored at 4°C for one day.

Cell Preparation

1. Harvest cells with a non-enzymatic cell dissociation solution, such as Versene™.
2. Wash cells once with HBSS (1X).
3. Resuspend the cells in stimulation buffer at the desired concentration. It is recommended to test 1,000-10,000 cells per assay.
4. Prepare a no-cell control consisting of stimulation buffer alone.

Test Compound Preparation

1. Prepare intermediate 2X or 4X dilution series of test compound(s) by serially diluting compound(s) across 12 wells of a polypropylene 96-well plate into stimulation buffer.

NOTE: It is recommended that a 12-point, half-log interval concentration-response curve be conducted at least in duplicate for an accurate estimation of the EC_{50} or IC_{50} value.

2. Alternatively, for hydrophobic DMSO-soluble test compounds, perform the initial dilutions in 100% DMSO, and then dilute the compound dilution series into stimulation buffer, resulting in a 2X (2% DMSO) or 4X (4% DMSO) dilution. Most cells can tolerate up to 1% DMSO in the 10 µl cell treatment step.

NOTE: The assay tolerance to DMSO must be established before conducting a test compound titration in DMSO vehicle. It is important to keep equal solvent concentrations between treated and untreated cells. In addition, when testing serial dilutions of compounds, the solvent concentrations should always remain constant across the dilution series.

ASSAY PROTOCOL

Assay Workflow

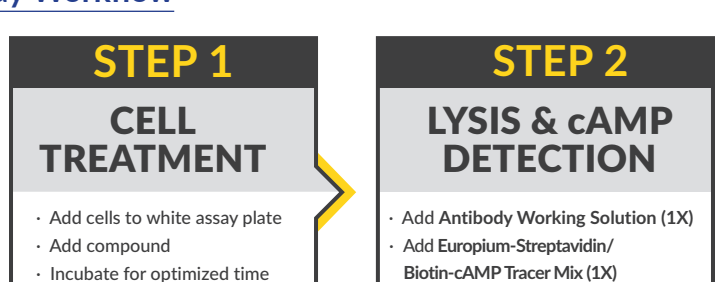


Figure 4. Assay workflow

Performing the Assay

The assay procedure assumes that both the cell density and stimulation conditions have been optimized during assay development as described in the Assay Optimization section (see page 10). NOTE: Optimal cell densities should be determined during assay development in cell titration experiments such that the measured signal falls within the IC_{10}-IC_{90} range of the cAMP standard curve.

Assays are performed in low-volume 384-well microplates at a total volume of 20 µl. Assays can be conducted in half-area 96-well plates or 1,536-well plates by adjusting the volumes of each assay component proportionally.

Pipetting protocols were developed for assays using suspension cells but the assay can be adapted easily for adherent cells.

It is recommended to include a cAMP standard curve each time a cAMP assay is run to verify the assay generates the expected S/B ratio and IC_{50} value and to convert the signal into cAMP levels.

Samples and standards MUST be assayed at least in duplicate (triplicate recommended) each time the assay is performed.

Cells and test compounds MUST be prepared in stimulation buffer that includes 0.5 mM IBMX.

Addition of the cAMP Detection Buffer (1X) lyses the cells. cAMP Detection Buffer (1X) MUST be used only for the preparation of Antibody Working Solution (1X) and the Eu-SA/Biotin-cAMP Tracer Mix (1X).

Do NOT premix the Antibody Working Solution (1X) and Eu-SA/Biotin-cAMP Tracer Mix (1X) in order to eliminate an addition step.

cAMP Standard Curve	G_s Agonist	G_s Antagonist	G_i Forskolin	G_i Agonist	G_i Antagonist
5 µl cAMP Standard (4X)	5 µl cell suspension				
5 µl stimulation buffer	5 µl agonist (2X)*	2.5 µl agonist (4X)*	5 µl forskolin (2X)*	2.5 µl forskolin (4X)*	2.5 µl forskolin/ agonist mix (4X)*
--	--	2.5 µl antagonist (4X)*	--	2.5 µl agonist (4X)*	2.5 µl antagonist (4X)*
Seal the plate and incubate for 30 minutes at room temperature					
5 µl Antibody Working Solution (1X)					
5 µl Eu-SA/Biotin-cAMP Tracer Mix (1X)					
Seal the plate and incubate 1 hour at room temperature**					
Remove plate seal and read plate on a TR-FRET-compatible microplate reader					

Table 3. Pipetting different cAMP assays

**The untreated cells receive the same volume of stimulation buffer and are incubated for the same amount of time and temperature as the treated cells.*

***Additional readings can be preformed for at least 18 hours without any negative effect on the assay performance.*

TR-FRET Plate Reader Settings

We recommend reading the TR-FRET assays at two wavelengths, detecting both the emission from the Eu chelate donor fluorophore at 615 nm and the acceptor fluorophore at 665 nm. Table 4, below, provides instrument settings to be used as guidelines.

	TR-FRET-compatible Plate Reader	
Parameter	**Flash lamp excitation**	**Laser excitation**
Excitation filter	320 nm (or 340 nm)	N/A
Emission filter	615 nm (or 620 nm)	615 nm (or 620 nm)
Delay time	90 µs	50 µs
Flash energy level	100% or High	100%
Number of flashes	100	20
Window (integration time)	300 µs	100 µs

Table 4. Recommended TR-FRET plate reader settings

ANALYSIS

Calculations

Because the TR-FRET signal is read in a time-resolved manner, background subtraction is usually not necessary.

1. TR-FRET data are typically calculated and presented ratiometrically using the following formula:

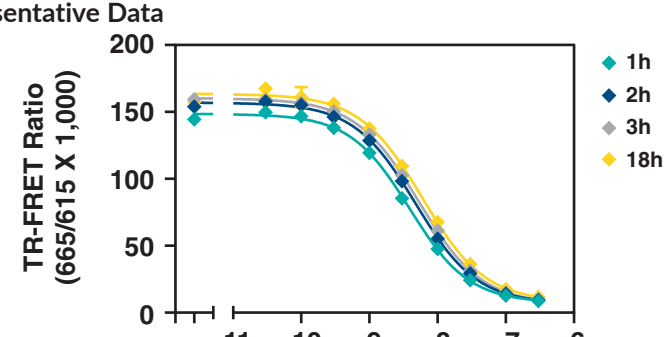
   [(665 nm/615 nm) x 1,000]

2. Calculate the TR-FRET ratio for each well. Alternatively, the signals at 665 nm can be used directly to analyze the data.
3. Create a standard curve by analyzing the data according to a nonlinear regression using the four-parameter logistic equation (sigmoidal dose-response curve with variable slope) and a $1/Y^2$ data weighting.

IMPORTANT: Convert the results from the reader (TR-FRET ratio or signals at 665 nm) into cAMP levels using the cAMP standard curve.

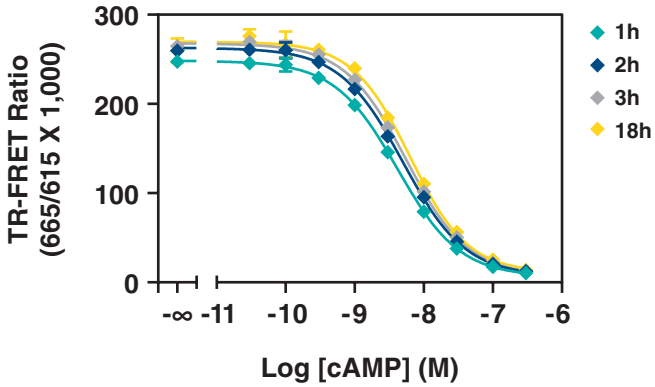
Performance Characteristics

Representative Data



Detection time (h)	1	2	3	18
IC_{50} (nM)	3.8	4.7	5.3	5.9
S/B	17	17	16	14

Figure 5. Typical nonlinear cAMP standard curve using lamp excitation Data represent the mean ± standard deviation of triplicate measurements (three wells) for each standard dilution. The assay working range is defined as IC_{10}-IC_{90}. The data was generated using a low-volume 384-well white plate read with an EnVision® Multilabel Plate Reader equipped with a TR-FRET option after 1, 2, 3, or 18 hour incubations. Please note that depending on the instrument and the lot of kit used, the level of counts and S/B ratio may slightly vary without affecting the assay robustness.



Detection time (h)	1	2	3	18
IC_{50} (nM)	4.1	4.9	5.5	6.3
S/B	24	21	21	18

Figure 6. Typical nonlinear cAMP standard curve using laser excitation Data represent the mean ± standard deviation of triplicate measurements (three wells) for each standard dilution. The assay working range is defined as IC_{10}-IC_{90}. The data was generated using a low-volume 384-well white plate read with an EnVision® Multilabel Plate Reader equipped with a TR-FRET option after 1, 2, 3, or 18 hour incubations. Please note that depending on the instrument and the lot of kit used, the level of counts and S/B ratio may slightly vary without affecting the assay robustness.

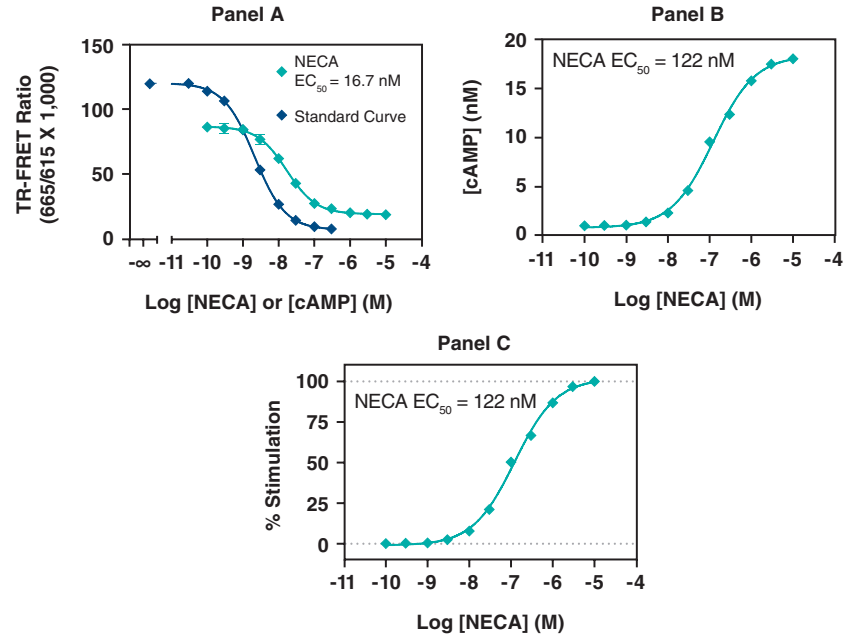


Figure 7. Agonist concentration-response curves in U266B1 cells U266B1 cells expressing endogenous G_s-coupled adenosine A_{2A} receptors were seeded at 2,000 cells per well. **Panel A:** The agonist response is plotted as the TR-FRET ratio (665/615 * 1,000) with the cAMP standard curve. The agonist produced signals within the working range of the standard curve. **Panel B:** The agonist response is plotted as the cAMP level to determine the EC_{50} value. **Panel C:** The same agonist response is plotted as % stimulation. *NOTE: IBMX is an antagonist of adenosine receptors and was therefore omitted from the stimulation buffer to improve assay sensitivity.*

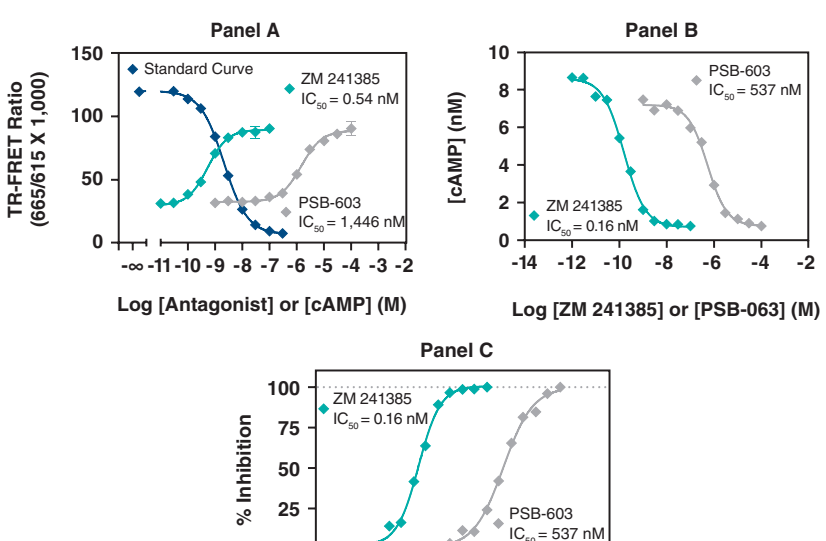


Figure 8. Antagonist concentration-response curves in U266B1 cells U266B1 cells expressing endogenous G_s-coupled adenosine A_{2A} receptors were seeded at 2,000 cells per well. **Panel A:** The antagonist responses are plotted as the TR-FRET ratio (665/615 * 1,000) with the cAMP standard curve. The antagonists produced signals within the working range of the standard curve. **Panel B:** The antagonist responses are plotted as the cAMP level to determine the IC_{50} values. **Panel C:** The same antagonist responses are plotted as % inhibition. *NOTE: IBMX is an antagonist of adenosine receptors and was therefore omitted from the stimulation buffer to improve assay sensitivity. NOTE: ZM 241385 is a potent antagonist of the adenosine A_{2A} receptor, while PSB-603 is a selective antagonist of the adenosine A_{2B} receptor.*

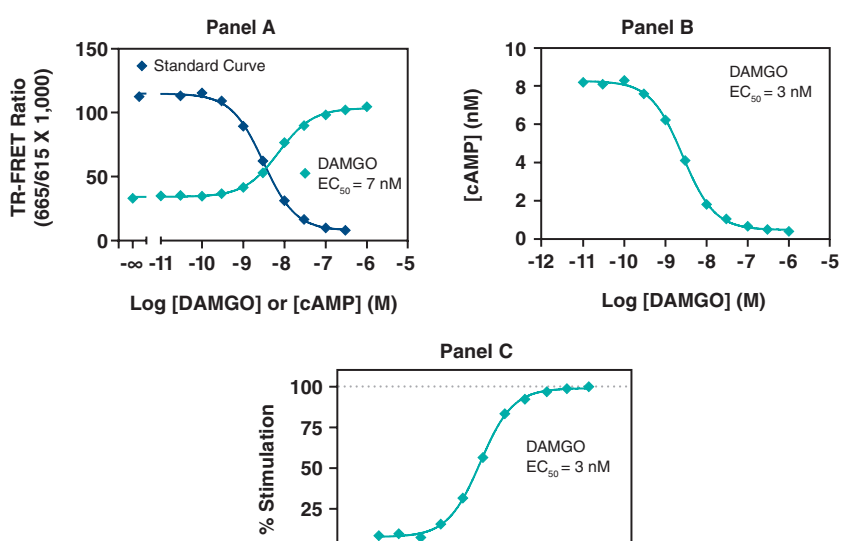


Figure 9. Agonist concentration-response curves in CHO cells CHO cells expressing G_i-coupled human μ-opioid receptors were seeded at 2,500 cells per well. **Panel A:** The agonist response is plotted as the TR-FRET ratio (665/615 * 1,000) with the cAMP standard curve. The agonist produced signals within the working range of the standard curve. **Panel B:** The agonist response is plotted as the cAMP level to determine the EC_{50} value. **Panel C:** The same agonist response is plotted as % stimulation.

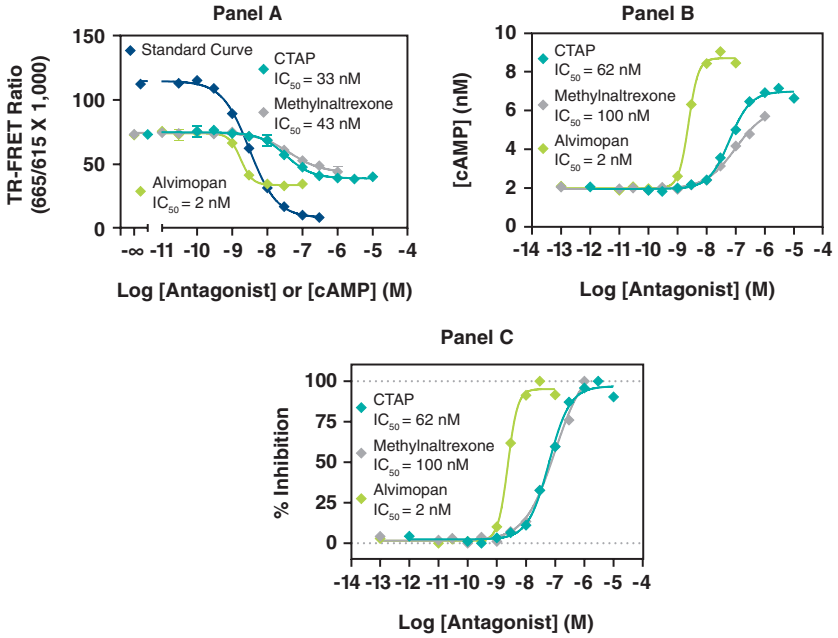


Figure 10. Antagonist concentration-response curves in CHO cells CHO cells expressing G_i-coupled human μ-opioid receptors were seeded at 2,500 cells per well. **Panel A:** The antagonist responses are plotted as the TR-FRET ratio (665/615 * 1,000) with the cAMP standard curve. The antagonists produced signals within the working range of the standard curve. **Panel B:** The antagonist responses are plotted as the cAMP level to determine the IC_{50} values. **Panel C:** The same antagonist responses are plotted as % inhibition.

RESOURCES

References

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2. Fimia, G.M. and Sassone-Corsi, P. Cyclic AMP signalling. *J. Cell Sci.* **114(Pt 11)**, 1971-1972 (2001).

NOTES

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