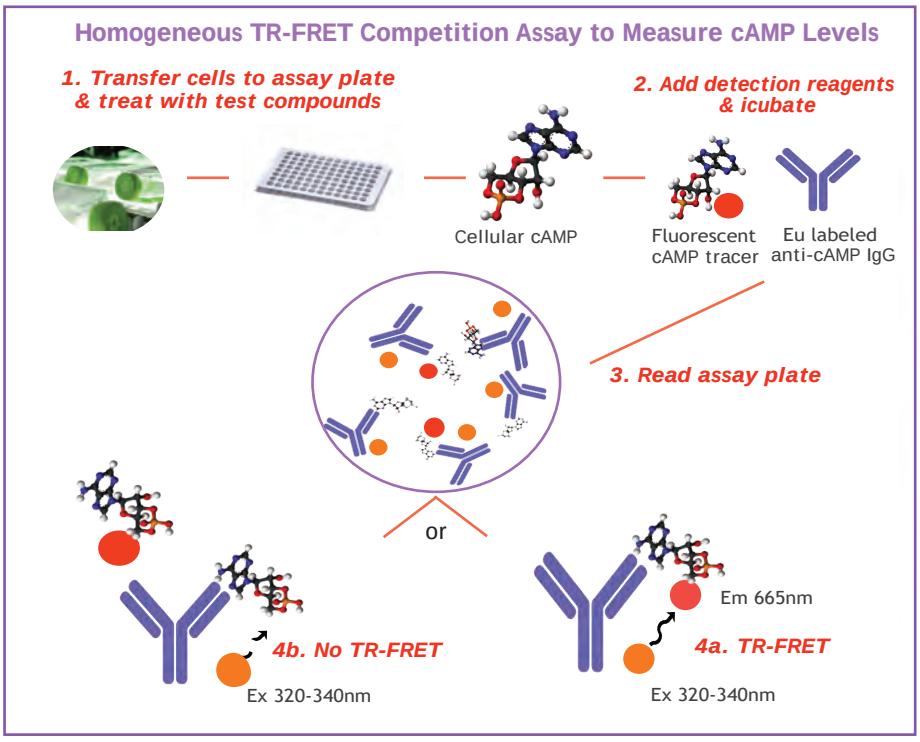
Cyclic AMP TR-FRET Kit

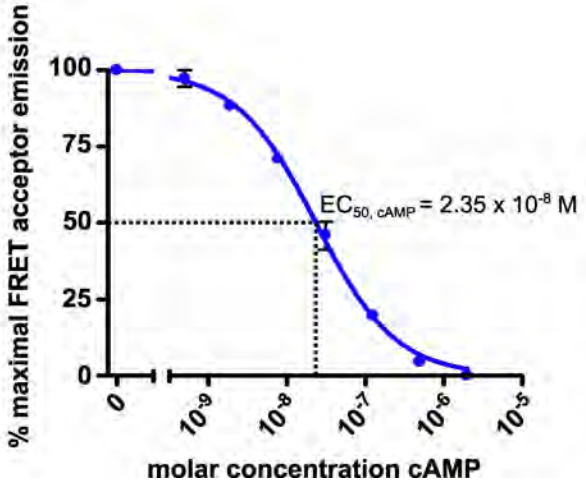
Columbia Biosciences develops and manufactures fluorescent proteins, antibody conjugates and, most recently, assay kits. Our easy-to-use Cyclic AMP Assay Kit is ideal for the measurement of intracellular cAMP and for small molecule drug screening.

cAMP TR-FRET Kit

The G protein-coupled receptor (GPCR) family of cell surface receptors comprises hundreds of proteins that are crucially involved in various physiological processes such as olfaction, neurotransmission, blood-brain barrier maintenance, kidney function, hormonal signaling, etc. The involvement of GPCRs in such diverse physiological processes and their activation by specific and unique ligands makes these proteins particularly attractive targets for drug development studies addressing GPCR-related diseases. A large proportion of GPCRs act by modulating the levels of cellular cAMP, a second messenger molecule responsible for the allosteric activation of GPCR-linked cell signaling cascades. Thus, assays that measure changes in cellular cAMP levels can be used to test acute GPCR activation or inhibition, thereby enabling the discovery of new therapeutic compounds that target specific GPCR activity.

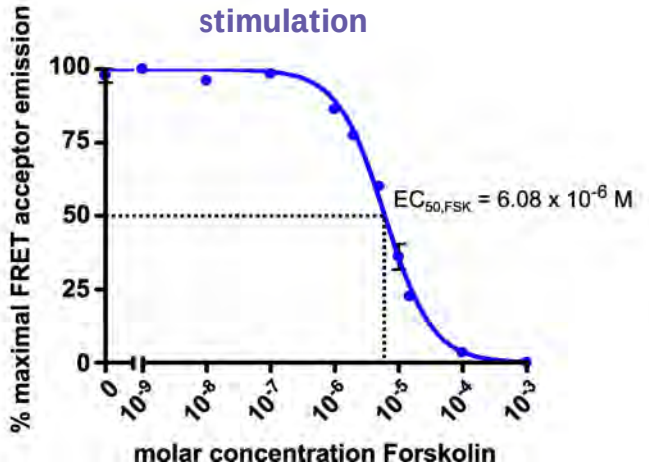


1. cAMP assay kit measurement of cAMP standards



1. An unlabeled cAMP standard was titrated to prescribed concentrations in triplicate into the wells of a 384-well microassay plate. Kit reagents were then loaded into wells according to kit protocol and plate was incubated in the dark for 1 hour at 25C. The fluorescence counts emitted by α-cAMP FRET donor (615 nm) and cAMP tracer (665 nm) were read per well on a TR-FRET plate reader. Fluorescence counts at 665 nm corresponding to each concentration of cAMP assayed were normalized to the maximal 665 nm signal measured in the absence of unlabeled cAMP for statistical analysis.

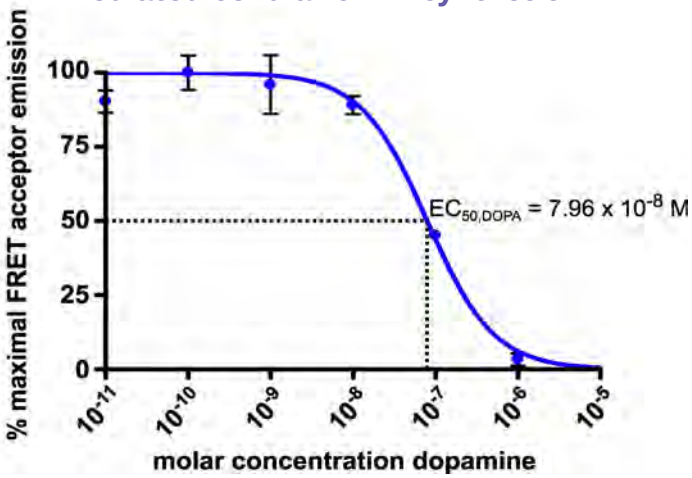
2. cAMP assay kit measurement of cellular cAMP levels downstream of adenylate cyclase stimulation



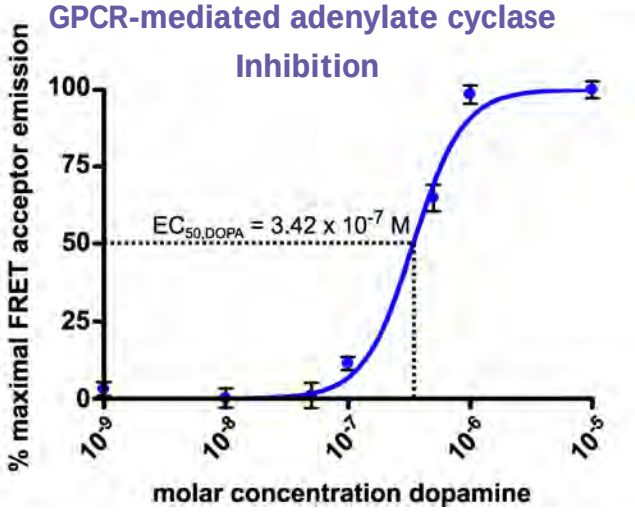
2. HEK293 cells were plated at a quantity of 6000 cells per well in a 384-well microassay plate. In triplicate, wells were then treated with increasing concentrations of Forskolin diluted in culture media for 30 minutes at 37C. Following treatment, kit reagents were added to each well according to kit protocol, and plate was incubated for 1 hour in the dark at 25C. Fluorescence emissions of α-cAMP FRET donor (615 nm) and cAMP tracer (665 nm) reagents were read per well on a TR-FRET plate reader, and 665 nm readings corresponding to each Forskolin treatment were normalized to maximal assay signal for subsequent graphing and statistical analysis.

3. HEK293 cells stably overexpressing the DRD1 G_S-coupled dopamine receptor were plated at a quantity of 6000 cells per well in a 384-well microassay plate. In triplicate, wells were then treated with increasing concentrations of dopamine diluted in culture media for 30 minutes at 37C. Following treatment, kit reagents were added to each well according to kit protocol, and plate was incubated for 1 hour in the dark at 25C. Fluorescence emissions of α-cAMP FRET donor (615 nm) and cAMP tracer (665 nm) reagents were read per well on a TR-FRET plate reader, and 665 nm readings corresponding to each dopamine treatment were normalized to maximal assay signal for subsequent graphing and statistical analysis.

3. cAMP assay kit measurement of GPCR-mediated cellular cAMP synthesis



4. cAMP assay kit measurement of GPCR-mediated adenylate cyclase Inhibition



4. HEK293 cells stably overexpressing the DRD2 G_i-coupled dopamine receptor were plated at a quantity of 6000 cells per well in a 384-well microassay plate. In triplicate, wells were then treated with increasing concentrations of dopamine diluted in cultured media for 30 minutes at 37C, after which the adenylate cyclase agonist Forskolin (10 μM) was added for an additional 30 minutes, 37C incubation. Following treatment, kit reagents were added to each well according to kit protocol, and plate was incubated for 1 hour in the dark at 25C. Fluorescence emissions of α-cAMP FRET donor (615 nm) and cAMP tracer (665 nm) reagents were read per well on a TR-FRET plate reader, and 665 nm readings corresponding to each dopamine treatment were normalized to maximal assay signal for subsequent graphing and statistical analysis.