cisbio Bioassays

Selecting a cyclic AMP kit for assaying GPCR target activation

G protein–coupled receptors (GPCRs) are one of the most popular target classes investigated in the drug-discovery process. With its selection of kits (cAMP femto, cAMP dynamic and cAMP HiRange), Cisbio allows detection of a broad range of cyclic AMP concentrations.

GPCR signaling pathway

Classically, the GPCR signaling pathway is considered to be a three-component system that involves a seven-transmembrane-domain receptor, a trimeric G-protein complex (Gα, Gβ, Gγ) and an effector. When activated, the receptor associates with the G-protein complex; this causes the exchange of GDP bound to Gα for GTP, followed by the dissociation of Gα-GTP from the complex. The activated subunit Gα can couple to downstream effectors to regulate the amounts of second messengers. Classically, each GPCR can activate only one G protein.

After activation, GPCRs carry information within cells through two signaling pathways: regulation of cAMP level or of intracellular Ca^{2+}, whose liberation is triggered by inositol-1,4,5-triphosphate (IP3). cAMP, calcium and IP3 are the second messengers commonly assessed to follow GPCR activation.

Drugs targeting GPCRs account for the majority of the best-selling drugs and about 40% of all prescription pharmaceuticals in the marketplace. An ever-growing interest in investigating GPCR targets in several therapeutic areas is expected, motivated by the desire to select a drug for new targets or a more selective drug targeting a specific pathway.

The assessment of cAMP with the homogeneous time-resolved fluorescence (HTRF) kits allows the implementation of functional assays to screen new drug candidates in high-throughput screening (HTS) conditions. Together with the IP-One kit for assessment of IP1, a downstream metabolite of IP3, Cisbio proposes a complete GPCR assay platform.

Here we describe the HTRF kits for cAMP detection.

Functional assays: cAMP accumulation

All Cisbio cAMP kits are based on a competitive immunoassay between native cAMP produced by cells and cAMP labeled with the acceptor dye d2. The two entities compete for binding to a cAMP-specific antibody labeled with cryptate (europium or terbium cryptate). The specific signal generated by fluorescence resonance energy transfer (FRET) is inversely proportional to the concentration of cAMP in the standard or sample (**Fig. 1a**).

Intracellular cAMP level measurement facilitates early and direct pharmacological characterization of compounds acting on G_i- or G_s-coupled receptors. The intracellular cAMP concentration is modulated by agonist or antagonist activity at the receptor level. In a direct, homogeneous assay compatible with HTS conditions, cells expressing the target of interest are incubated with the compound. The assay

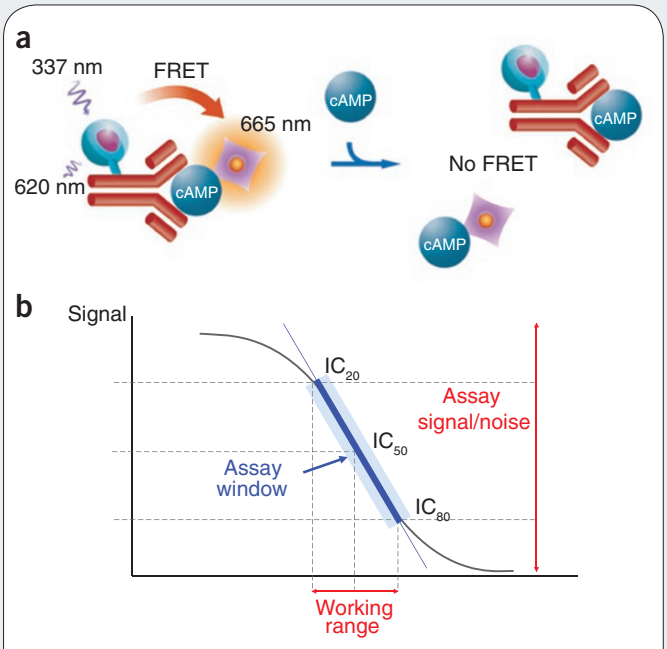

Figure 1 | Assay principle. (**a**) In the absence of cellular cAMP, the europium or terbium cryptate donor (cyan and magenta) conjugated to the antibody (red rods) is in close proximity to the d2 acceptor conjugated to the cAMP. FRET occurs after excitation at 337 nm, and long-lived fluorescence is emitted at 665 nm for the FRET acceptor and 620 nm for the donor. Free cAMP dissociates this binding and disrupts FRET dose-dependently (**b**).

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APPLICATION NOTES

Table 1 | The working range of the Cisbio's cAMP kits

HTRF kits	HTRF cryptate	IC_{20} (nM)	IC_{50} (nM)	IC_{80} (nM)
cAMP femto kit	Europium cryptate	0.33	1.76	9.4
cAMP femto Tb kit	Terbium cryptate			
cAMP dynamic kit	Europium cryptate	0.91	4.07	18.1
cAMP HiRange kit	Europium cryptate	4.27	22.8	121

The working range is defined as the cAMP concentration between 20 and 80% inhibitory concentration (IC_{20} and IC_{80}). In this range, the signal variation is linearly proportional to the cAMP concentration.

compares basal cAMP in the absence of GPCR stimulation with cAMP level reached after stimulation with a drug of interest.

cAMP kits to cover a broad range of assay conditions

Cisbio offers four kits covering a broad range of working cAMP concentrations (**Table 1**). The kits are intended for the direct, quantitative determination of cAMP and facilitate early, direct pharmacological characterization of compounds acting on G_i- or G_s-coupled receptors.

How to select a cAMP assay

In a cell-based assay, the intracellular cAMP level depends not only on the agonist or antagonist activity but also on the cellular model used. The technical criteria for evaluating the quality of an assay are the sensitivity, the assay signal-to-noise ratio, the working range (**Fig. 1b**), the robustness (*Z'* factors), the miniaturization and the number of steps.

In a cell-based assay, the cAMP produced within a well determines the working range of the assay, so several biological criteria influence the selection of a cAMP assay. The cell density is different for suspension versus adherent cells and is an important parameter to optimize. The maximal cell density for adherent cells depends on the surface of the wells, which often becomes a limiting parameter in HTS. Receptor expression level and coupling efficiency for recombinant cells directly affect cAMP regulation. Measurement of G_i-coupled receptor stimulation requires a cAMP assay capable of discriminating a slight variation of cAMP concentration; that is, having a good signal-to-noise ratio. The pharmaceutical class of drug under investigation also has a bearing on the specifications required for the cAMP assay. For antagonism assays, sensitivity is often required to detect a slight variation of cAMP concentration (**Table 2**).

Table 2 | Effect of agonist and antagonist on the cAMP level

Drug	Mode of action	Effect for G_i-coupled receptor	Effect for G_s-coupled receptor
Agonist	Produces the same maximal effect as the endogenous ligand.	In the presence of an agonist, the cAMP level decreases.[a]	In the presence of an agonist, the cAMP level increases.
Antagonist	Blocks receptor response by competing with agonists or inverse agonists.	In the presence of an antagonist, the effect of the agonist is inhibited, and the cAMP level increases.[b]	In the presence an agonist, the antagonist reduces the cAMP level.[b]

[a]To perform an agonist assay, the adenlyate cyclase must be first directly activated by a specific agent such as forskolin. [b]The effect of an antagonist is measured in the presence of an agonist.

Conclusion

Because of the variability of the biological material and the pharmacological compounds acting on G_i- or G_s-coupled receptors, a selection of cAMP assay kits is necessary to cover a broad range of concentrations.

The homogeneous cAMP assay kits offered by Cisbio are compatible with robust (*Z'* factors), miniaturized, high-throughput screens in either 384 or 1,536 wells. These cAMP accumulation assays have been applied to the investigation of G_s- and G_i-coupled GPCRs and can be used with recombinant, nonrecombinant or native receptors.