cAMP Chemiluminescent Immunoassay Kit

Catalog nos. C10557, C10558

Table 1. Contents and storage information.

Material	C10557 (2-plate size)	C10558 (10-plate size)	Storage	Stability
Assay/lysis buffer (Component A)	25 mL	130 mL	• 2–6˚C • DO NOT FREEZE	When stored as directed, kit components are stable for at least 6 months.
Conjugate dilution buffer (Component B)	10 mL	50 mL		
cAMP-AP conjugate (Component C)	100 µL	500 µL		
Anti-cAMP antibody (Component D)	14 mL	70 mL		
cAMP standard (Component E)	2 mL	10 mL		
Wash buffer (Component F)	500 mL	2 × 1,000 mL		
CSPD® substrate/Sapphire-II™ enhancer (Component G)	25 mL	130 mL	• 2–6˚C • Protect from light • DO NOT FREEZE	
Pre-coated microplates (Component H)	1 each	5 each	• 2–6˚C • Dessicate • DO NOT FREEZE	

Number of assays: Sufficient material is supplied for 192 (Cat. no. C10557) or 960 (Cat. no. C10558) assays based on the protocol below.

Introduction

The cAMP Chemiluminescent Immunoassay kit is designed for the rapid and sensitive quantitation of 3',5'-cyclic AMP (cAMP) in extracts prepared from mammalian cells cultured in microwell plates without the need for sample acetylation or standards.[1] The cAMP Chemiluminescent Immunoassay is a competitive immunoassay format that incorporates an alkaline phosphatase (AP)-labeled cAMP conjugate, a highly specific anti-cAMP antibody, pre-coated microplates, cAMP standard, and CSPD® substrate with Sapphire-II™ luminescence enhancer to generate glow light emission kinetics. Light signal intensity is inversely proportional to the cAMP level in the sample or standard preparation, and is measured in a luminometer 30 minutes after substrate addition. The simple assay format and glow light emission kinetics achieved with cAMP Chemiluminescent Immunoassay kit reagents provide an ideal assay system for automated high-throughput screening applications. The kit includes all required reagents and pre-coated microplates.

Cyclic AMP (cAMP) is an important second messenger in many signal transduction pathways, linking activation of cell surface membrane receptors to intracellular responses, and ultimately, to changes in gene expression. cAMP is synthesized by plasma membrane-bound adenylate cyclase, which is coupled to transmembrane receptors for numerous hormones, neurotransmitters, and other signaling molecules by heterotrimeric G-proteins. Upon ligand binding to G-protein coupled receptors (GPCRs), the intracellular receptor domain interacts with a G-protein, which then dissociates and activates adenylate cyclase activity, resulting in an increase in the concentration of intracellular cAMP. Subsequently, cAMP activates cAMP-dependent protein kinases (protein kinase A), which phosphorylate specific substrate proteins including enzymes, structural proteins, transcription factors, and ion channels. In addition, both hormone-mediated activation and inhibition of adenylate cyclase has been demonstrated.

The CSPD® substrate supplied with the cAMP Chemiluminescent Immunoassay kit is a chemiluminescent 1,2-dioxetane enzyme substrate that allows a wide dynamic assay range of cAMP quantitation in a variety of ultrasensitive assay systems. The cAMP Chemiluminescent Immunoassay kit provides a highly sensitive, simple-to-use chemiluminescent assay solution that is amenable for use in automated, high-throughput screening applications.

Applications

The cAMP Chemiluminescent Immunoassay kit is designed for quantitating cellular cAMP for functional assays of receptor activation, and has been used with established cell lines for functional measurements with endogenous receptors,[1–4] cell lines with exogenously expressed ligand receptors,[5,6] primary cells,[7,8] and tissues[9,10] in response to treatment with the appropriate ligands, as well as for receptor characterization,[11,12] orphan receptor ligand identification,[13] and the characterization of novel chimeric receptors.[14] In addition, it can be used for high throughput screening assays[1,15] for compounds which stimulate or interfere with these signal transduction pathways.

Before Starting

Materials Required but Not Provided

- Mammalian cells and appropriate growth media
- 96-well tissue culture-treated microplates
- Microscope to determine the extent of cell lysis
- Microplate luminometer
- *Optional:* serum-free media containing IBMX (3-isobutyl-1-methylxanthone) phosphodiesterase inhibitor at 0.1–1mM final concentration

Caution

Component A is irritating to eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. If swallowed seek medical advice immediately and show this container or label. Wear suitable protective clothing, gloves, and eye/face protection. It is harmful to aquatic organisms, and may cause long-term adverse effects in the aquatic environment.

Component D is harmful if swallowed. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). This material and its container must be disposed of as hazardous waste. Wear suitable protective clothing and gloves.

Components F and G are irritating to eyes and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection.

General Guidelines

Cell Culture

- The assay/lysis buffer (Component A) supplied with the cAMP Chemiluminescent Immunoassay kit is optimized for use with adherent or non-adherent mammalian cell lines.
- You can use a wide variety of cell types of tissue origin, as well as primary cells (platelets, adipocytes, hepatocytes, lymphocytes, osteoblasts, fibroblasts) and cell lines. Cells expressing endogenous ligand receptors (SK-N, AtT20/D, NCI-H716, T84, A549, THP-1) and transfected (exogenous) ligand receptors (CHO, HEK293) have been successfully used with the cAMP chemiluminescent immunoassay systems.
- You can use wide range of cell densities (typically 1,000 to 50,000 cells/well, depending on the cell type), but we recommend empirically determining the cell density that provides the optimal experimental response.
- You may culture your cells and perform the experiments in tissue culture-treated microplates of your choice. After preparing the samples, transfer the cell lysates to the pre-coated assay plate provided with the kit.

Cell Lysis

- The detergent in the assay/lysis buffer (Component A) has a low cloud point of ~20°C, where it separates into an aqueous and a detergent phase giving it a cloudy appearance. Before use, mix the assay/lysis buffer well to ensure correct detergent concentration, as the detergent will settle to the bottom.
- You may observe some remaining cell structure after cell lysis. Since cAMP is a small molecule, and is soluble, permeabilization of cells will enable its release from cells. It is not necessary to observe "complete" lysis to the extent that cells are no longer visible to ensure adequate results. You can increase incubation time, if desired.

Luminometers

- The light signal obtained with the cAMP Chemiluminescent Immunoassay kit exhibits "glow" light emission kinetics, and has a very broad emission spectrum, with an emission maximum of ~470 nm. There are no special instrumentation requirements for the cAMP Chemiluminescent Immunoassay kit assays, other than using a good quality microplate luminometer. We recommend using a single-mode luminometer or a multi-mode detection instrument set for luminescence measurement to measure light emission from 96- or 384-well microplates.
- You cannot use a microplate fluorometer or spectrophotometer to measure chemiluminescence unless there is a luminometer reading mode on the instrument.
- Reagent injectors are not required.
- If instrument is a multi-mode instrument, do **not** use excitation or emission filters as the use of filters can possibly block some of the light signal and reduce sensitivity.
- Make sure the luminometer is warmed up before starting your measurements.

cAMP assay

- For optimal performance, use the reagents supplied with the kit; the use of buffers not supplied with the kit may adversely affect assay performance.
- Perform all incubation steps at room temperature unless noted otherwise.
- To minimize plate edge effects (*i.e.*, lower or higher signal levels in outer wells of the plate compared to middle wells) caused by uneven evaporation of the reagents or temperature fluctuation of the plate platform in instrument, cover or seal the plate during incubations.
- You can read the assay plates at 30 minutes. If you are running multiple plates together and comparing them to each other, take care to determine the optimal time after substrate addition to read the plates. Peak relative light units (RLU) in some cases may not be reached until 45 to 60 minutes after substrate addition, and will be maintained for several hours. When comparing results from multiple plates we advise reading plates once they have reached the peak RLU.

Preparing Standards

The cAMP standard (Component E) has a concentration of 1 mM (1,000 pmol/µL). Follow the protocol below to obtain a cAMP standard concentrations in range of 0.006–6,000 pmol per 60 µL for the standard curve. Prepare diluted cAMP standards **fresh** each time.

1.1 Prepare seven serial 1:10 dilutions of the cAMP standard in assay/lysis buffer (Component A) according to Table 2.

Table 2. cAMP standard dilutions for the standard curve.

Dilution	cAMP standard (Component E)	Assay/lysis buffer (Component A)	cAMP concentration*
1	30 µL of cAMP standard	270 µL	6,000
2	30 µL of Dilution 1	270 µL	600
3	30 µL of Dilution 2	270 µL	60
4	30 µL of Dilution 3	270 µL	6
5	30 µL of Dilution 4	270 µL	0.6
6	30 µL of Dilution 5	270 µL	0.06
7	30 µL of Dilution 6	270 µL	0.006
Blank	0 µL	300 µL	0

* cAMP concentration in pmol per 60 µL.

Preparing Lysates for cAMP Assay

You can use a wide range of cell densities (typically 1,000–50,000 cells per well), but we recommend that you optimize your experimental conditions by determining the cell density that provides the optimal experimental response.

Adherent Cells

2.1 Plate 100 µL of cells per well in a 96-well tissue-culture treated microplate, and incubate at the appropriate growth conditions for up to several days until cells reach the desired density.

2.2 *Optional*: If desired, remove media and replace with 90 µL per well of serum-free media containing 0.1–1mM IBMX (3-isobutyl-1-methylxanthone) phosphodiesterase inhibitor.

2.3 Treat your cells for the appropriate response time by adding 5 µL of inducer/compound per well.

2.4 Remove media from wells and add 60 µL of assay/lysis buffer (Compound A) into each well.

Note: You may prepare the lysates from adherent cells in the presence or absence of culture media.

2.5 Incubate at 37°C for 5–30 minutes or until cells are lysed. Determine the extent of lysis by observing the cells under a microscope.

Suspension Cells

3.1 Plate 25 µL of cells per well in a 96-well tissue-culture treated microplate, and incubate at the appropriate growth conditions for up to several days until cells reach the desired density.

3.2 Treat your cells for the appropriate response time by adding 5 µL of inducer/compound per well.

3.3 Add an equal volume (*i.e.*, 30 µL) of assay/lysis buffer (Compound A) into each well.

3.4 Incubate at 37°C for 5–30 minutes or until cells are lysed. Determine the extent of lysis by observing the cells under a microscope.

cAMP Assay

Make sure you have read the **General Guidelines** above before commencing your experiments.

4.1 Dilute the cAMP-AP conjugate (Component C) 1:100 with conjugate dilution buffer (Component B). Prepare 4 mL of diluted conjugate (40 µL of cAMP-AP conjugate + 3.96 mL of conjugate dilution buffer) per 96-well plate. Dilute only sufficient conjugate for immediate use.

4.2 Add 60 µL/well of cAMP standard dilutions (prepared in step 1.1) or lysate (prepared in step 2.5 or 3.4) to the wells of the pre-coated microplate (Component H).

4.3 Add 30 µL/well of diluted cAMP-AP (prepared in step 4.1) to the wells of the pre-coated assay plate containing the cAMP standard dilutions and the lysate. Mix by repeated pipetting or on a plate shaker.

4.4 Add 60 µL of anti-cAMP antibody (Component D) into each well containing the cAMP standard or lysate and diluted cAMP-AP mixture. Mix by repeated pipetting or on a plate shaker.

4.5 Incubate for 1 hour at room temperature.

Note: This assay was designed for use in automated robotic systems without shaking. However, results may improve with shaking.

4.6 Remove the solution from wells and wash with wash buffer (Component F). Repeat the wash step five more times.

4.7 Add 100 µL of CSPD® Substrate/Sapphire-II™ Enhancer solution into each well and incubate for 30 minutes.

4.8 Measure the chemiluminescence signal in a microplate luminometer for 1 second per well or as appropriate for the instrument used.

Expected Results

cAMP Chemiluminescent Immunoassay is a competitive ELISA, so there is an inverse correlation between cAMP concentration in the sample and the assay signal intensity. Low levels of cAMP result in a high luminescence intensity, while a high concentration of cAMP results in a low signal.

Measurement of the cAMP standards will give a sigmoidal standard curve, which is best fit with a weighted four-parameter logistic curve. Figure 1 shows an example of the standard curve.

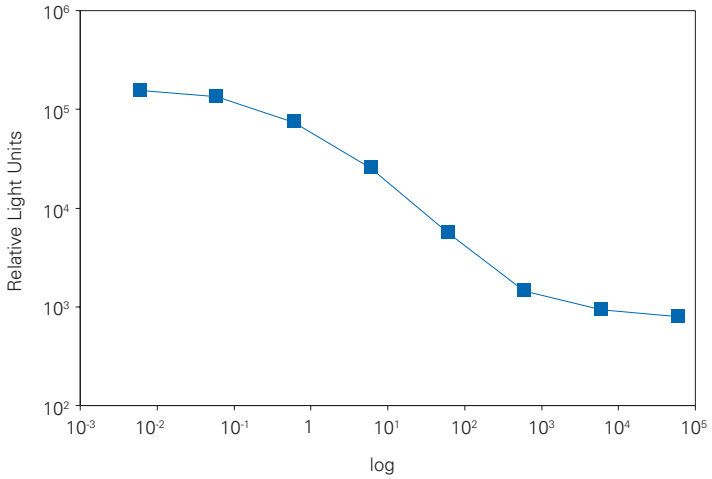

Figure 1. cAMP Standard curve. Assays were performed with the cAMP Standard as described above. Measurements were made on the TR717™ microplate luminometer.

Appendix

Cross Reactivity Specifications

The cAMP Chemiluminescent Immunoassay kit utilizes a highly sensitive and specific chemiluminescent substrate, CSPD®, which exhibits very low cross-reactivity with other adenosine-containing or cyclic nucleotides as summarized in Table 3 below.

Table 3. Cross-reactivity of the cAMP Chemiluminescent Immunoassay kit.

Nucleotide	Cross-reactivity*
cAMP	100%
AMP	0.15%
ADP	0.03%
ATP	0.15%
cGMP	0.02%
cUMP	0.012%
cIMP	0.15%
cTMP	0.06%
CTP	0.002%
GMP	0.005%
GTP	0.2%

*Cross-reactivity relative to cAMP.

References

1. J Biomol Screen 5, 239 (2000); **2.** Molecular Pharmacology 73, 1371 (2008); **3.** J Med Chem 51, 1831 (2008); **4.** J Biol Chem 280, 4048 (2005); **5.** Molecular Endocrinology (USA) 21, 700 (2007); **6.** J Biochem 139, 543 (2006); **7.** J Immunol 174, 1073 (2005); **8.** Kidney International 71,738 (2007); **9.** J Clin Invest 112, 398 (2003); **10.** J Pharmacol Exp Therapeutics 317, 676 (2006); **11.** J Biol Chem 279, 19790 (2004); **12.** J Pharmacol Exp Therapeutics 304, 1217 (2003); **13.** Nature 429, 188 (2004); **14.** Proc Natl Acad Sci USA 101, 1508 (2004); **15.** Nuc Acid Res 31, e130 (2003).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
C10557	cAMP Chemiluminescent Immunoassay Kit *2-plate size*	1 kit
C10558	cAMP Chemiluminescent Immunoassay Kit *10-plate size*	1 kit

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