#8019 Store at -20°C

Cyclic AMP XP® Chemiluminescent Assay Kit

☑ 1 Kit
(96 assays)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Description: The Cyclic AMP XP® Chemiluminescent Assay Kit is a competition enzyme-linked immunoassay used to determine cAMP levels in cells or tissues of interest. In this assay, cAMP found in the test sample competes with a fixed amount of HRP-linked cAMP for binding to an anti-cAMP XP® Rabbit mAb immobilized onto a 96-well plate. Following washing to remove excess sample cAMP and HRP-linked cAMP, chemiluminescent reagent is added for signal development. Because of the competitive nature of this assay, the magnitude of light emission, measured in relative light units (RLU), is inversely proportional to the quantity of sample cAMP. Measurement of light emmision using the cAMP Standard allows calculating the absolute amount of cAMP in a sample of interest.

Background: Cyclic adenosine 3',5'-monophosphate (cAMP) is an important second messenger involved in many signal transduction pathways in different cell types of numerous species (1-3). In mammalian cells, this important molecule is produced by adenylyl cyclases (AC). Extracellular stimuli such as neurotransmitters, hormones, chemokines, lipid mediators, and drugs can modulate AC activity to increase or decrease cAMP production by binding to a large number of transmembrane G protein-coupled receptors (4). The degradation of cAMP to AMP is catalyzed by phosphodiesterases that are regulated by intracellular nucleotide concentrations, phosphorylation, or binding of Ca^{2+}/calmodulin and other regulatory proteins (5). A set of diverse molecules, including cAMP-dependent protein kinase (PKA), cyclic nucleotide-gated ion channels, and exchange proteins that are activated by cAMP (EPAC), mediate downstream cAMP signaling (6,7). cAMP modulates various biological processes including metabolism, differentiation, cardiac cell functions, neuronal signaling, cell adhesion, and immune functions (5-7).

Specificity/Sensitivity: The immunoreactivity of this kit was tested against the following: ADP, AMP, ATP, cAMP, cGMP, cIMP, cTMP, CTP, GDP, GMP, and GTP. Relatively minor cross-reactivity was observed with cGMP and cIMP, with 10 fold higher sensitivity for cAMP compared to either cGMP or cIMP. No cross-reactivity was observed with any of the other factors tested. Kit sensitivity, as shown in Figure 1, demonstrates a dynamic range of 0.2 to 12 nM of cAMP. Changes in cellular cAMP levels following specific treatment with forskolin is shown in Figure 2 (CHO cells).

Product Includes	Item #	Kit Quantity	Color	Storage Temp
cAMP Rabbit mAb Coated Microwells*	50715	96 assays		4°C
cAMP-HRP Conjugate	55401	5.5ml	Red	-20°C
cAMP Standard (2.4 uM)	38720	0.5ml		-20°C
Luminol/Enhancer Solution	84850	3ml		RT
Stable Peroxide Buffer	42552	3ml		RT
Sealing Tape	54503	2 sheets		RT
ELISA Wash Buffer (20X)	9801	10ml		4°C
Cell Lysis Buffer (10X)	9803	15ml		-20°C

Low volume microplate *12 8-well modules – Each module is designed to break apart for 8 tests.

Background References:

(1) Serezani, C.H. et al. (2008) *Am J Respir Cell Mol Biol* 39, 127-32.
(2) Beavo, J.A. and Brunton, L.L. (2002) *Nat Rev Mol Cell Biol* 3, 710-8.
(3) Kopperud, R. et al. (2003) *FEBS Lett* 546, 121-6.
(4) Kamenetsky, M. et al. (2006) *J Mol Biol* 362, 623-39.
(5) Cheng, J. and Grande, J.P. (2007) *Exp Biol Med (Maywood)* 232, 38-51.
(6) Holz, G.G. et al. (2006) *J Physiol* 577, 5-15.
(7) Taylor, S.S. et al. (2008) *Biochim Biophys Acta* 1784, 16-26.

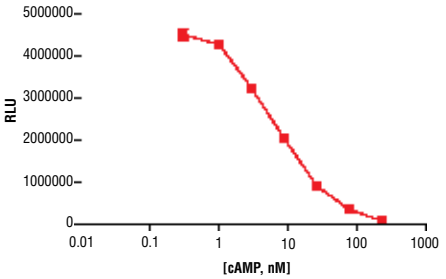

Figure 1: cAMP Standard was diluted in 1X Cell Lysis Buffer #9803 and samples were assayed following the Cyclic AMP XP® Chemiluminescent Assay Kit protocol. This standard curve is for demonstration purposes only; users should generate a standard curve for each sample set in order to accurately determine cAMP concentration.

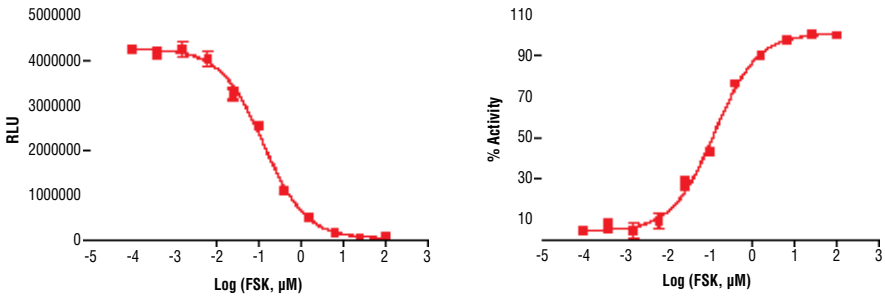

Figure 2: Treatment of CHO cells with Forskolin (FSK) #3828 increases cAMP concentration as detected by Cyclic AMP XP® Chemiluminescent Assay Kit #8019. CHO cells were seeded at 4x10⁴ cells/well in a 96-well plate and incubated overnight. Cells were pretreated with 0.5 mM IBMX for 30 minutes prior to forskolin treatment (15 minutes) and lysed with 1X Cell Lysis Buffer #9803. The light emission values (left) and percentage of activity (right) are shown above. The percentage of activity is calculated as follows: % activity=100x[(RLU-RLU_{basal})/(RLU_{max}-RLU_{basal})], where RLU is the sample relative light unit, RLU_{max} is the light emission at maximum stimulation (i.e., high forskolin concentration), and RLU_{basal} is the light emission at basal level (no forskolin). Forskolin directly activates adenylyl cyclases and increases cellular cAMP concentration. IBMX is a non-specific inhibitor of cAMP and cGMP phosphodiesterases and promotes accumulation of cAMP and cGMP in cells.

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Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine
Dg—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Chemiluminescent ELISA Protocol

A Reagent Preparation

1. Bring all microwell strips to room temperature before use.
2. Prepare 1X Wash Buffer by diluting 20X Wash Buffer (included in each kit) in Milli-Q or equivalently purified water.
3. Dilute the 10X Cell Lysis Buffer #9803 to 1X in Milli-Q or equivalently purified water. 1 mM phenylmethylsulfonyl fluoride (PMSF) should be added fresh each time. This buffer can be stored at 4°C for short-term use (1–2 weeks).

B Preparing Cell Lysates

1. Plate cells of interest in 96-well plate (typically between 6-100 X 10^3 cells/well) and incubate overnight under appropriate cell culture conditions.
2. Rinse cells with 200 µl warm PBS, then add test compounds in serum free mediums and incubate cells for the desired time period.
3. Rinse cells twice with 200 µl ice cold PBS, and then add 100 µl/well 1X lysis buffer, keep cells on ice for 5 to 10 minutes.

NOTE: If cell debris is observed it can be removed by brief centrifugation of the plate and transfer of the clear lysates to a new 96 well plate.

C Assay

1. Bring all kit components to room temperature.
2. Make cAMP standard in the 1X Cell Lysis buffer: Take 50 µl of the cAMP standard (2.4 µM) and add it to 450 µl diluent to get 240 nM cAMP. Perform a 1:3 serial dilution of this standard to get 80 nM, 26.7 nM, 8.9 nM, 3.0 nM, 1.0 nM, 0.3 nM,and 0 nM. The diluent without cAMP will serve as the 0 nM cAMP.

Note: The standard curve is used to calculate the absolute amount of cAMP in the sample and is necessary for each assay.

3. Add 25 µl of the HRP-linked cAMP solution and 25 µl sample to the cAMP assay plate. Cover the plate and incubate at room temperature for 3 hours on a horizontal orbital plate shaker.
4. Discard plate contents and wash wells 4 times with 200 µl /well of 1X Wash Buffer. Make sure to discard all liquid after each wash but do not allow wells to completely dry.
5. Prepare working solution by mixing equal parts Luminol/Enhancer Solution and Stable Peroxide Buffer.
6. Add 50 µl of the Working Solution to each well.

Use a plate-based luminometer to measure Relative Light Units (RLU) at 425nM within 1-10 minutes following addition of the substrate. Optimal signal intensity is achieved when read within 10 minutes.

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