Store at -20°C

#4339

Cyclic AMP XP® Assay Kit

☑ 1 Kit
(96 assays)

Cell Signaling TECHNOLOGY®

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rev. 03/15/18

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

Description: The Cyclic AMP XP® 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 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, HRP substrate TMB is added to develop color. Because of the competitive nature of this assay, the magnitude of the absorbance for this developed color is inversely proportional to the quantity of sample cAMP. Measurement of absorbance using the cAMP Standard allows calculating the absolute amount of cAMP in a sample of interest.

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 treatments are shown in Figure 2 (CHO cells) and Figure 3 (293 cells).

Products Included	Item #	Kit Quantity	Color	Storage Temp
cAMP Rabbit mAb Coated Microwells*	74746	96 assays		4°C
cAMP-HRP Conjugate	97796	11ml	Red	-20°C
cAMP Standard (2.4 uM)	38720	1ml		-20°C
TMB Substrate	7004	11ml		4°C
STOP Solution	7002	11ml		4°C
Sealing Tape	54503	2 sheets		4°C
ELISA Wash Buffer (20X)	9801	10ml		4°C
Cell Lysis Buffer (10X)	9803	15ml		-20°C

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

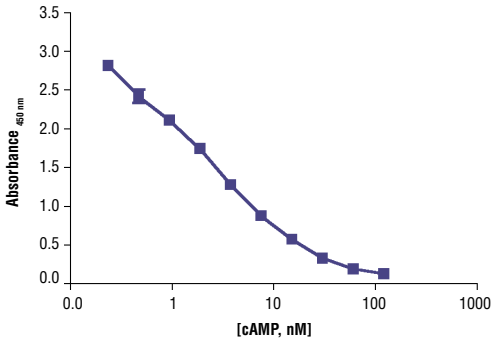

Figure 1: cAMP Standard was diluted in 1X Cell Lysis Buffer #9803 and samples were assayed following the Cyclic AMP XP® Assay Kit protocol. This standard curve is for demonstration purposes only; users should generate a standard curve for each sample set in order to accurately determine cAMP concentration.

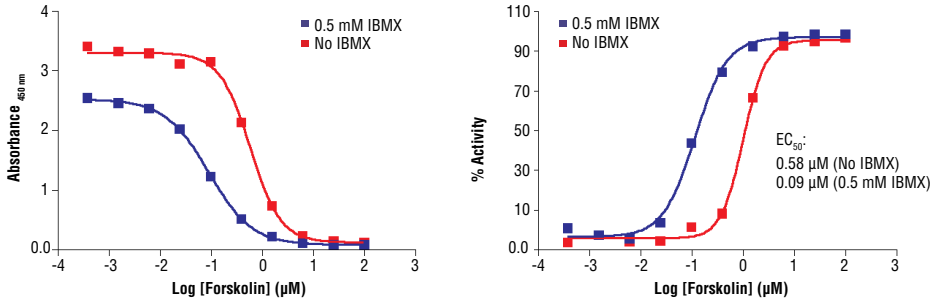

Figure 2: Treatment of CHO cells with Forskolin #3828 increases cAMP concentration as detected by Cyclic AMP XP® Assay Kit #4339. CHO cells were seeded at 4x10⁴ cells/well in a 96-well plate and incubated overnight. Cells were either left untreated or pretreated with 0.5 mM IBMX for 30 minutes prior to forskolin treatment (15 minutes) and lysed with 1X Cell Lysis Buffer #9803. The absorbance values (left) and percentage of activity (right) are shown above. The percentage of activity is calculated as follows: % activity=100X[(A-A_{basal})/(A_{max}-A_{basal})], where A is the sample absorbance, A_{max} is the absorbance at maximum stimulation (i.e., high forskolin concentration), and A_{basal} is the absorbance 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.

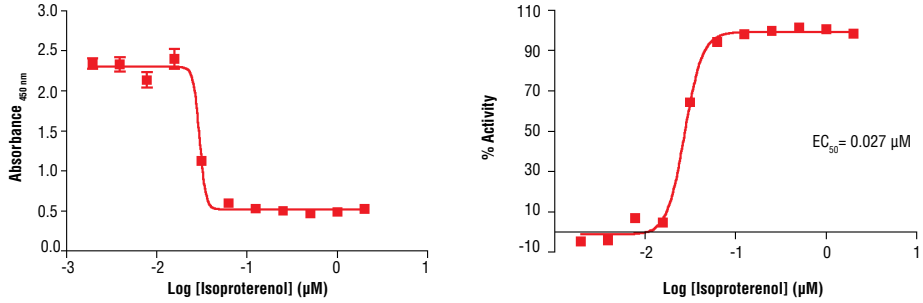

Figure 3: Treatment of 293 cells with isoproterenol increases the cAMP concentration as detected by Cyclic AMP XP® Assay Kit #4339. 293 cells were seeded at 3x10⁴ cells/well in a 96-well plate and incubated overnight. Cells were pretreated with 0.5 mM IBMX for 30 minutes prior to isoproterenol treatment (3 minutes) and lysed with 1X Cell Lysis Buffer #9803. The absorbance values (left) and percentage of activity (right) are shown above. The percentage of activity is calculated as follow: % activity=100X[(A-A_{basal})/(A_{max}-A_{basal})], where A is the absorbance of the sample, A_{max} is the absorbance at maximum stimulation (i.e., high isoproterenol concentration), and A_{basal} is the absorbance at basal level (no isoproterenol). Isoproterenol is a β-adrenoceptor agonist and activates β-2 adrenergic receptors (ADRB2) that are endogenously expressed on 293 cells. Activation of ADRB2 then leads to activation of adenylyl cyclase and synthesis of cAMP as its second messenger.

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).

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.

cAMP XP® Assay Kit 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 Cell Lysate Preparation

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 0nM. 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 50 µl of the HRP-linked cAMP solution and 50 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. Add 100 µl TMB substrate.
6. Incubate for 30 minutes at room temperature.
   Note: Watch the color as it being developed since it may be necessary to stop the reaction before 30 minutes.
7. Add 100 µl STOP solution.
8. Measure absorbance at 450 nm (for optimal results, read the plate within 30 minutes after adding STOP solution).