Cyclic AMP XP® Assay Kit

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, cTMP, CTP, GDP, GMP and GTP. Relatively minor cross-reactivity was observed with cGMP and cTMP, with 10 fold higher sensitivity for cAMP compared to either cGMP or cTMP. 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).

Note: This kit contains mixed storage components. Please store this entire kit at -20°C for long term storage. Upon first use, please allow components to thaw and then store each component as indicated on individual component labels.

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

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 absorbance of the sample, A_basal is the absorbance at basal level (no forskolin), A_max is the absorbance at maximum stimulation (i.e., high forskolin concentration), and A_max-basil is the absorbance at basal level (no forskolin). Forskolin directly activates adenyl 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 follows: % activity=100X[(A-A_basal)/(A_max-A_basal)], where A is the absorbance of the sample, A_basal is the absorbance at maximum stimulation (i.e., high isoproterenol concentration), and A_max is the absorbance at basal level (no isoproterenol). Isoproterenol is a β-adrenergic agonist and activates β2-adrenergic receptors (ADRB2) that are endogenously expressed on 293 cells. Activation of ADRB2 then leads to activation of adenyl 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 Ca2+/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:
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).
Material Safety Data Sheet (MSDS) for cGMP Assay Kits

I. Identification:
Product name: cAMP Glycolysis Assay Kit
Product code: 428-30-0
CAS number: 5457-43-6

II. Composition/Information on Ingredients:
Preparation Name: cAMP Glycolysis Assay Kit
GDA: None

III. Hazard Identification:
This product is for Research Use Only. Please see the attached material safety data sheet for important information to ensure safe handling of this product.

IV. First Aid Measures for Hazardous Ingredient:
Inhalation: Remove to fresh air. If breathing is difficult, get medical attention.
Ingestion: May be harmful if swallowed. May cause respiratory irritation. Get medical attention.
Skin Contact: Wash with soap and water for at least 15 minutes. If skin irritation develops or persists, get medical attention.
Eye Contact: Flush with water for at least 15 minutes. Get medical attention.

V. Fire Fighting Measures:
Flash Point: Not applicable.
Autogenous Temperature: Not applicable.

VI. Accidental Release Measures:
Wear appropriate personal protective equipment. In case of an accidental spill, transfer contaminated absorbent to a closed chemical waste container for disposal. Wash spill area with soap and water for at least 15 minutes.

VII. Handling and Storage:
Store at 4°C with gentle shaking. Avoid contact with eyes, skin, and clothing. Wash thoroughly after handling. Air pollution or repeated exposure may result in respiratory irritation.

VIII. Exposure Controls/Personal Protective Equipment:
Ventilation System: Local exhaust ventilation is recommended.
Eye Protection: Polypropylene safety glasses.
Respiratory Protection: Not applicable.

IX. Physical and Chemical Properties:
Appearance: White crystalline powder.
Odor: None.
Solubility (water): Soluble in water.
Stability: Stable under ordinary conditions.

X. Ecological Information:
Toxicity: Not known.
Bioaccumulation: Not known.
Persistence: Not known.

XI. Toxicological Information:
Acute Toxicity: Not established.
Mutagenicity: Not established.
Teratogenicity: Not established.
Reproductive Toxicity: Not established.
Hazardous Substance: None.
Hazardous Waste: None.

XII. Biological Information:
Toxicology: No data available.

XIII. Disposal Considerations:
Dispose of contents/label in accordance with local, state, and federal environmental regulations.

XIV. Transport Information:
DOT: None.
IMDG: None.

XV. Regulatory Information:
Hazardous Substance: 20X LumiGLO

XVI. Other Information:
This substance is not classified as a hazardous substance according to EC Directive 67/548/EEC.

For further information, please refer to the Material Safety Data Sheet for cAMP Glycolysis Assay Kit, which is available from Cell Signaling Technology.

Material Safety Data Sheet (MSDS) for cAMP-HRP Conjugate

I. Identification:
Product name: cAMP–HRP Conjugate
Product code: 4360, 8020
CAS number: 5457-56-9

II. Composition/Information on Ingredients:
Preparation Name: cAMP–HRP Conjugate
GDA: None

III. Hazard Identification:
This product is for Research Use Only. Please see the attached material safety data sheet for important information to ensure safe handling of this product.

IV. First Aid Measures for Hazardous Ingredient:
Inhalation: Immediately flush eyes water for at least 15 minutes. Get medical attention.
Ingestion: May be harmful if swallowed. May cause respiratory irritation. Get medical attention.
Skin Contact: If skin irritation develops or persists, get medical attention.
Eye Contact: Flush eyes with water for a minimum of 15 minutes. Get medical attention.

V. Fire Fighting Measures:
Flash Point: Not applicable.
Autogenous Temperature: Not applicable.

VI. Accidental Release Measures:
Wear protective clothing and self-contained breathing apparatus to prevent inhalation of vapor or mist. Transfer contaminated absorbent to a closed chemical waste container for disposal. Wash spill area with soap and water for at least 15 minutes.

VII. Handling and Storage:
Store at -20°C to -80°C. Avoid prolonged or repeated exposure. Avoid contact with eyes, skin, and clothing. Wash thoroughly after handling. Air pollution or repeated exposure may result in respiratory irritation.

VIII. Exposure Controls/Personal Protective Equipment:
Ventilation System: Local exhaust ventilation is recommended.
Eye Protection: Polypropylene safety glasses.
Respiratory Protection: None.

IX. Physical and Chemical Properties:
Appearance: Lyophilized powder.
Odor: None.
Solubility (water): Soluble in water.
Molecular weight: 34,290
Melt/Freezing Point: 10°C to 15°C
Specific Optical Activity: None

X. Ecological Information:
Toxicity: Not known.
Bioaccumulation: None.
Persistence: None.

XI. Toxicological Information:
Acute Toxicity: Not known.
Acute Dermal irritation: None.
Harmful if swallowed.
Harmful if inhaled.

XII. Disposal Considerations:
Dispose of contents/label in accordance with local, state, and federal environmental regulations.

XIII. Transport Information:
DOT: Not dangerous goods.
IMDG: Not dangerous goods.

XIV. Regulatory Information:
Hazardous Substance: 20X LumiGLO

XV. Regulatory Information:
Hazardous Substance: 20X LumiGLO

XVI. Other Information:
This substance is not classified as a hazardous substance according to EC Directive 67/548/EEC.

For further information, please refer to the Material Safety Data Sheet for cAMP-HRP Conjugate, which is available from Cell Signaling Technology.
I. Identification:
Product name: TMB Substrate Solution
Product Catalog: 704X
Manufacturer: Cell Signaling Technology
575 Boston Post Rd
Beverly, MA 01915 USA
1-978-867-2400 FAX
1-978-578-6737 Emergency TEL

II. Composition/Information on Ingredients:
Ingredient Name | CAS# | %
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Proprietary Ingredient | CAUTION! Do not use this material undiluted. Exposure to this material may cause severe eye irritation, respiratory irritation, and skin sensitization. This material should be handled only by personnel trained in hazardous materials handling. A respiratory protection program is required. This product is flammable. Use caution when handling, breathing, and open flame equipment should not be used near this material. Keep out of reach of children. 

III. Hazard Identification:
Inhaled 2-fluorobenzidine (CAS: 68-13-3) is classified as "carcinogenic" to humans (Group 1). This product is also contained in the TMB Substrate Solution, also flammable, and explosive. 

IV. First Aid Measures:
Inhalation: Remove to fresh air. If breathing is difficult, get medical attention. 
Ingestion: If ingested, seek medical attention immediately. 
Skin Exposure: Wash skin with soap and water. 
Eye Exposure: Wash out eyes with plenty of water for at least 15 minutes. Get medical attention immediately. 

V. Fire Fighting Measures:
Fire Fighting: Water spray, dry chemical, alcohol foam, or carbon dioxide. 

VI. Accidental Release Measures:
Wear protective clothing and self-contained breathing apparatus to prevent exposure. 

VII. Handling and Storage:
Store at 2°C – 8°C in tightly closed container. 

VIII. Exposure Controls/Personal Protection Equipment:
Skin Protection: Wear protective clothing and self-contained breathing apparatus to prevent exposure. 

IX. Physical And Chemical Properties:
Physical Form: Liquid 
Odor: None 
Odor Threshold Value: Unknown 
Specific Gravity: 1.1 (d20/4°C) 
Solubility: Soluble in water 

X. Stability and Reactivity:
Stability: Stable under normal conditions of storage and handling. 
Reactivity: Inorganic in nature. 

XI. Toxicological Information:
Inhalation LC50: >720mg/m3/1H, rat 
Skin Sensitization: No data available 

XII. Ecological Information:
Toxicity to Aquatic Life: Not available 
Toxicity to Soil microorganisms: No data available 
Toxicity to Earthworms: No data available 

XIII. Disposal Considerations:
Disposal of uncollectable or contaminated material must be in accordance with local, state, and federal environmental regulations. Contact a licensed professional waste disposal service to dispose of this material.

XIV. Transportation Information:
Proper Shipping Name: Not available 
Proper Calculation: Not available 

XV. Regulatory Information:
OSHA Hazard Classification: Not available 

XVI. Other Information:
This product is not intended for use in humans. The use of this product is the responsibility of the research scientist. The use of this product as intended by the manufacturer is safe for use. 

Material Safety Data Sheet (MSDS) for TMB Substrate Solution
Rev. 5/19/10