Cyclic GMP XP® Assay Kit

#4360 Store at -20°C

☑ 1 Kit
(96 assays)

Cell Signaling TECHNOLOGY®

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

Description: The Cyclic GMP XP® Assay Kit is a competition enzyme-linked immunoassay used to determine cGMP levels in cells or tissues of interest. In this assay, cGMP found in test sample competes with a fixed amount of HRP-linked cGMP for binding to an anti-cGMP XP® Rabbit mAb immobilized onto a 96-well plate. Following washing to remove excess sample cGMP and HRP-linked cGMP, 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 cGMP. Measurement of absorbance using the cGMP Standard allows calculating the absolute amount of cGMP 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. Minor cross-reactivity was observed with cIMP, with over 100 fold higher sensitivity for cGMP compared to 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 2 to 200 nM of cGMP. Changes in cellular cGMP levels following specific treatments are shown in Figure 2 and Figure 3 (low passage RFL-6 cells).

Product Includes	Item #	Kit Quantity	Color	Storage Temp
cGMP Rabbit mAb Coated Microwells*	10551	96 tests		4°C
cGMP-HRP Conjugate	51283	11ml	Red	-20°C
cGMP Standard (5 uM)	30440	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

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

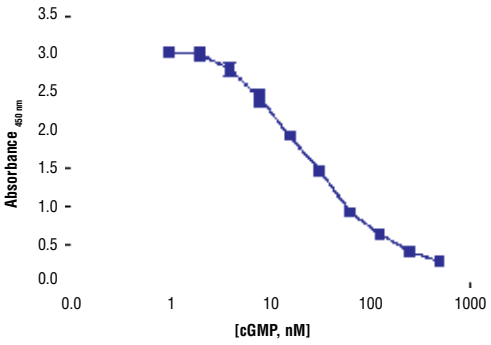

Figure 1: cGMP Standard was diluted in 1X Cell Lysis Buffer #9803 and samples were assayed following the Cyclic GMP XP® Assay Kit protocol. This standard curve is for demonstration purposes only; users should generate a standard curve for each sample set in order to accurately determine cGMP concentration.

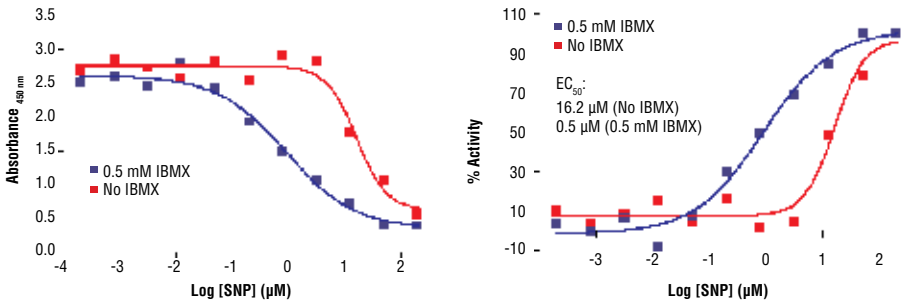

Figure 2: Treatment of RFL-6 cells with sodium nitroprusside (SNP) increases cGMP concentration as detected by Cyclic GMP XP® Assay Kit #4360. RFL-6 cells were seeded at 2x10^5 cells/well in a 12-well plate and incubated overnight. Cells were either left untreated or pretreated with 0.5 mM IBMX for 15 minutes prior to SNP treatment (30 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 SNP concentration), and A$_{basal}$ is the absorbance at basal level (no SNP). SNP is a nitric oxide donor that directly activates soluble guanylyl cyclases and increases cellular cGMP concentration. IBMX is a non-specific inhibitor of cAMP and cGMP phosphodiesterases that promotes accumulation of cAMP and cGMP in cells.

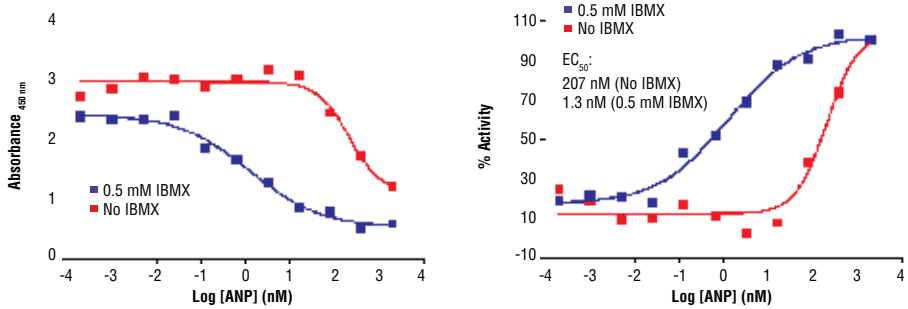

Figure 3: Treatment of RFL-6 cells with atrial natriuretic peptide (ANP) increases cGMP concentration as detected by Cyclic GMP XP® Assay Kit #4360. RFL-6 cells were seeded at 2x10^5 cells/well in a 12-well plate and incubated overnight. Cells were either left untreated or pretreated with 0.5 mM IBMX for 15 minutes prior to ANP treatment (30 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 ANP concentration), and A$_{basal}$ is the absorbance at basal level (no ANP). ANP is a ligand that binds and activates membrane bound guanylyl cyclases to increase cellular cGMP concentration.

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.

Background: Cyclic guanosine 3',5'-monophosphate (cGMP) is a critical and multifunctional second messenger molecule involved in many signal transduction pathways in different cell types of almost all species (1). Intracellular cGMP is generated from GTP by guanylyl cyclase (GC) and degraded through phosphodiesterase (PDE) hydrolysis (1,2). Two distinctive families of GC have been identified: soluble guanylyl cyclases (sGC) that are nitric oxide-responsive and cell membrane-bound, particulate guanylyl cyclases (pGC) that respond to diverse extracellular agonists including peptide hormones, bacterial toxins and free radicals (2,3). Phosphodiesterases form a superfamily of 11 isoforms with different specificity to both cyclic adenosine 3',5'-monophosphate (cAMP) and cGMP (4). Cyclic GMP regulates cellular physiology by activating cGMP-dependent kinase, modulating cGMP-dependent ion channels or transporters, and altering its own hydrolytic degradation by phosphodiesterase (PDE) (1,4). Because of the diversity of its effectors, cGMP plays an important role in regulating various pathological and physiological processes, such as vascular smooth muscle motility, intestinal fluid and electrolyte homeostasis, and retinal phototransduction (1,5).

Background References:

(1) Domek-Łopacińska, K. and Strosznajder, J.B. (2005) *J Physiol Pharmacol* 56 Suppl 2, 15-34.

(2) Lucas, K.A. et al. (2000) *Pharmacol Rev* 52, 375-414.

(3) Potter, L.R. et al. (2006) *Endocr Rev* 27, 47-72.

(4) Matsumoto, T. et al. (2003) *J Smooth Muscle Res* 39, 67-86.

(5) Rybalkin, S.D. et al. (2003) *Circ Res* 93, 280-91.

cGMP 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 cGMP standard in the 1X Cell Lysis buffer: Take 50 µl of the cGMP standard (5 µM) and add it to 450 µl diluent to get 500 nM cGMP. Perform a 1:3 serial dilution of this standard to get 166.7 nM, 55.6 nM, 18.5 nM, 6.2 nM, 2.1 nM, 0.7 nM and 0 nM. The diluent without cGMP will serve as the 0 nM cGMP.
   Note: The standard curve is used to calculate the absolute amount of cGMP in the sample and is necessary for each assay.
3. Add 50 µl of the HRP-linked cGMP solution and 50 µl sample to the cGMP 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).