

Cyclic GMP XP[®] Chemiluminescent Assay Kit



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✓ 1 Kit
(96 assays)

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

Description: The Cyclic GMP XP[®] Chemiluminescent 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 the 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, 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 cGMP. Measurement of light emission using the cGMP Standard allows calculating the absolute amount of cGMP in a sample of interest.

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, and 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 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).

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 (low passage RFL-6 cells).

Product Includes	Item #	Kit Quantity	Color	Storage Temp
cGMP Rabbit mAb Coated Microwells*	32140	96 tests		4°C
cGMP-HRP Conjugate	60309	5.5ml	Red	-20°C
cGMP Standard (5 µM)	30440	0.5ml		-20°C
Luminol/Enhancer Solution	84850	3ml		RT
Stable Peroxide Buffer	42552	3ml		RT
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.

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.

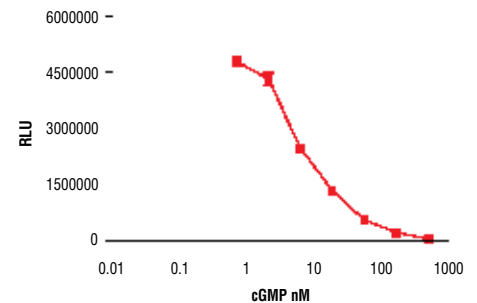


Figure 1: cGMP Standard was diluted in 1X Cell Lysis Buffer #9803 and samples were assayed following the Cyclic GMP XP[®] Chemiluminescent Assay Kit protocol. This standard curve is for demonstration purposes only; users should generate a standard curve for each sample set in order to accurately determine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

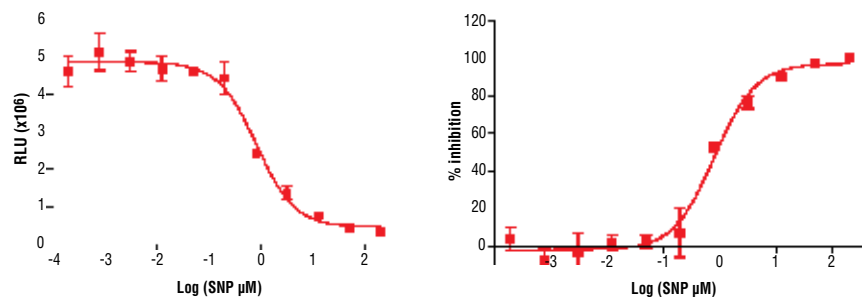


Figure 2: Treatment of RFL-6 cells with sodium nitroprusside (SNP) increases cGMP concentration as detected by Cyclic GMP XP[®] Chemiluminescent Assay Kit #8020. RFL-6 cells were seeded at 2x10⁵ cells/well in a 12-well plate and incubated overnight. Cells were either left untreated or pretreated with 0.5 mM IBMX for 15 minutes prior to SNP treatment (30 minutes) and lysed with 1X Cell Lysis Buffer #9803. The 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 relative light unit, RLU_{max} is the light emission at maximum stimulation (i.e., high SNP concentration), and RLU_{basal} is the light emission 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.

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 10³ 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 160.7 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 25 µl of the HRP-linked cGMP solution and 25 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. 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 425 nM within 1–10 minutes following addition of the substrate. Optimal signal intensity is achieved when read within 10 minutes.