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HitHunter® cGMP Assay

EFC Chemiluminescent Detection

User Manual: 90-0039 Series

Simple Solutions for Complex Biology

CONTENTS

NOTES:

NOTES:

LEGAL SECTION

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INTENDED USE

The HitHunter® cGMP assay measures cGMP in cell lysates from cells induced in suspension in a microtiter plate.

TECHNOLOGY PRINCIPLE

Enzyme fragment complementation (EFC) technology is based on splitting the *E. coli* β-galactosidase (β-gal) into two fragments, a large protein fragment (enzyme acceptor, EA) and a small peptide fragment (enzyme donor, ED). These fragments are inactive separately, but in solution, they rapidly recombine to form an active enzyme that hydrolyzes luminescent or fluorescent substrates to produce an easily detectable signal.

cGMP from cell lysates competes for antibody binding against labeled cGMP (ED-cGMP conjugate). Unbound ED-cGMP is free to complement EA to form active enzyme by EFC, which subsequently hydrolyzes luminescent substrate. The amount of signal generated is proportional to the amount of cGMP.

STORAGE CONDITIONS

Upon arrival, store reagents at -20°C. The kit is stable until the expiry date indicated on the outer kit box label. Thaw reagents at room temperature before use, and after thawing, store reagents for up to 1 month at 2-8°C. For longer term storage, aliquots of all the components may be re-frozen at -20°C. The reagents can be thawed and frozen for a TOTAL of 3 times without loss in performance.

NOTES:

DATA ANALYSIS

Average the raw RLU obtained from the wells. Plot the standard curve using a 4 parameter best-fit analysis.

REPRESENTATIVE DATA

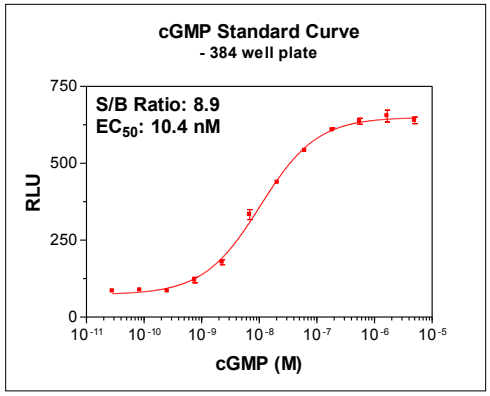

Figure 1. A representative standard curve is illustrated in the graph below. Typical Z' factor for the standard curve is 0.75.

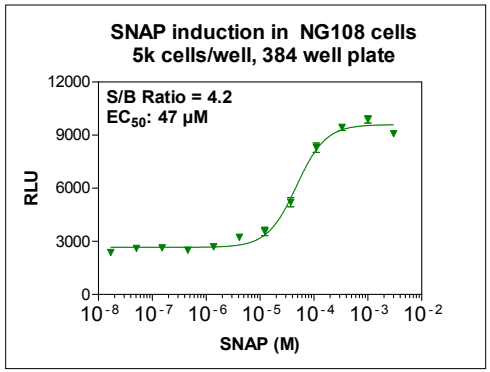

Figure 2. NG108 cells (10,000 cells/well) were induced with S-Nitroso-N-acetylpenicillamine (SNAP, Sigma N3398) in the presence of 1 mM IBMX for 30 minutes and assayed.

MATERIALS PROVIDED

HitHunter® cGMP assay kit contains:

Product Ordering Code		90-0039	90-0039M	90-0039L	90-0039 XL
# of test points		800	2,400	10,000	40,000
		384 well (volume in mL)			
Item	Description	Volume (mL per kit)			
1	cGMP Lysis Buffer	4	3 x 4	50	200
2	cGMP EA Reagent	8	3 x 8	100	400
3	cGMP ED Reagent	8	3 x 8	100	400
4	cGMP Antibody	4	3 x 4	50	200
5	cGMP Standard	1.0	3 x 1.0	13	52
6a 6b 6c	Substrate Reagent 2 Substrate Reagent 1 CL Substrate Diluent	0.48 2.4 9.1	3 x 0.48 3 x 2.4 3 x 9.1	6 30 114	24 120 456

NOTE:
cGMP Lysis Buffer (pH 6.9) contains 10 mM phosphate, 10 mM NaCl, 1 mM IBMX, lysing agent, and other proprietary components.

MATERIALS NOT PROVIDED

The following additional materials are required but not provided:

Equipment	Materials
• 96 or 384 well white plate • Pipettes and tips • Microtiter plate luminometer or CCD imager	• Serum free culture media with or without phenol red • IBMX (Sigma #I5879 or #I7018) • PBS (Sigma# D8537)

TIPS FOR OPTIMAL PERFORMANCE

1. Protect the reagents from light when possible and cover the microplate with foil or a black plate during all incubations.
2. Recommended controls:
   a) Basal level: measure cGMP in untreated cells
   b) EFC background: substitute PBS for ED reagent to obtain background EFC signal.
3. Addition of reducing agents, oxidizers such as Sodium nitroprusside (SNP), chelating agents, reducing agents, some detergents, or changes in pH may adversely affect assay performance.

REAGENT PREPARATION

Thaw reagents completely and equilibrate to ambient room temperature before use.

cGMP Standard - Dilute the cGMP Standard (6.0×10^{-5} M) to prepare (12) 3-fold serial dilutions in polypropylene tubes using PBS as the diluent. Use PBS for the zero standard. The final system concentration of cGMP in the standard curve ranges from 2.82×10^{-11} to 5.0×10^{-6} M.

cGMP Antibody/Lysis Mix – Gently mix 1 part cGMP Lysis Buffer and 1 part cGMP Antibody Reagent, to generate cGMP Lysis Buffer-Antibody Mix. cGMP Antibody/ Lysis mix is stable for 4 hours at room temperature.

cGMP ED Reagent – Ready to use.

cGMP EA Reagent – Ready to use.

Substrate Reagent – Gently mix Substrate Reagent 2, Substrate Reagent 1, and Substrate Diluent in the following ratio:

1 part Substrate Reagent 2: 5 parts Substrate Reagent 1: 19 parts Substrate Diluent.

For entire kit use, transfer the entire contents of Substrate Reagent 2 and Substrate Reagent 1 into the Substrate Diluent bottle. Gently mix by inversion. The mixture is stable for 24 hours at 2-8°C.

Cell Preparation – Harvest cells and resuspend cells in PBS or media at the desired concentration. Initially, a cell titration should be performed to establish the optimum cell concentration each specific cell line in the cGMP assay. We recommend 5,000, 10,000, and 20,000 cells per well of a 384-well plate as a starting point.

NOTE:
The cell lysis buffer contains 1 mM IBMX.

ASSAY PROCEDURE

Assay each standard in triplicate. The following procedure is for a 384 well and 96-half well plate formats.

Condition	Standard curve	Agonist
Step 1: Standard/Cells	15 µL Standard	10 µL Cells
Step 2: Agonist	-	5 µL Agonist
NA		**Incubate at 37°C for 30 minutes***
Step 3: Antibody/Lysis mix	10 µL cGMP Antibody/Lysis mix	
Step 4: ED Reagent	10 µL cGMP ED Reagent	
Incubate 60 minutes at room temperature		
Step 5: EA Reagent	10 µL cGMP EA Reagent	
Incubate 30 minutes at room temperature		
Step 6: Substrate	15 µL Substrate Reagent	
60 minutes incubation at room temperature		
Read on a luminescent reader at 1sec/well or 90-second exposure on a CCD-imager		

***The incubation time for the agonist may differ and should be optimized for each cell line.