



## CGMP KITS

Part # 62GM2PEG & 62GM2PEH

Test size#: 500 tests (62GM2PEG) and 10,000 tests (62GM2PEH) - assay volume: 20  $\mu$ L

Revision: #07 of September 2023

Store at: 2-8°C (62GM2PEG); 2-8°C (62GM2PEH)

For research use only. Not for use in diagnostic procedures.

### ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of cGMP in cell supernatants, cell lysates or buffered samples and offers a fast alternative to ELISA.

The detection principle of this kit is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1, cGMP is detected in a competitive assay by using anti cGMP antibody labeled with Europium cryptate (donor), and cGMP labeled with d2 (acceptor).

When the dyes are in close proximity, the excitation of the donor with a light source (laser or flash lamp) triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665 nm). The cGMP present in the sample competes with the binding between the two HTRF detection solutions and thereby prevents FRET from occurring. The specific signal is inversely proportional to the cGMP concentration.

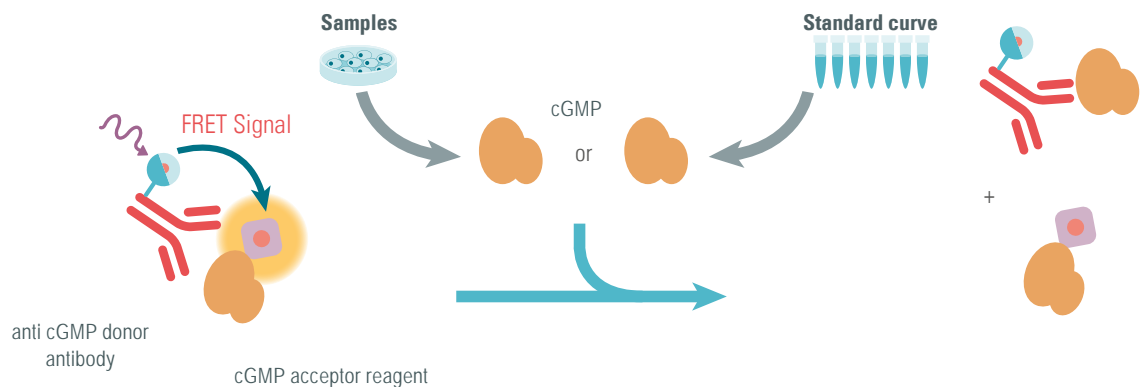
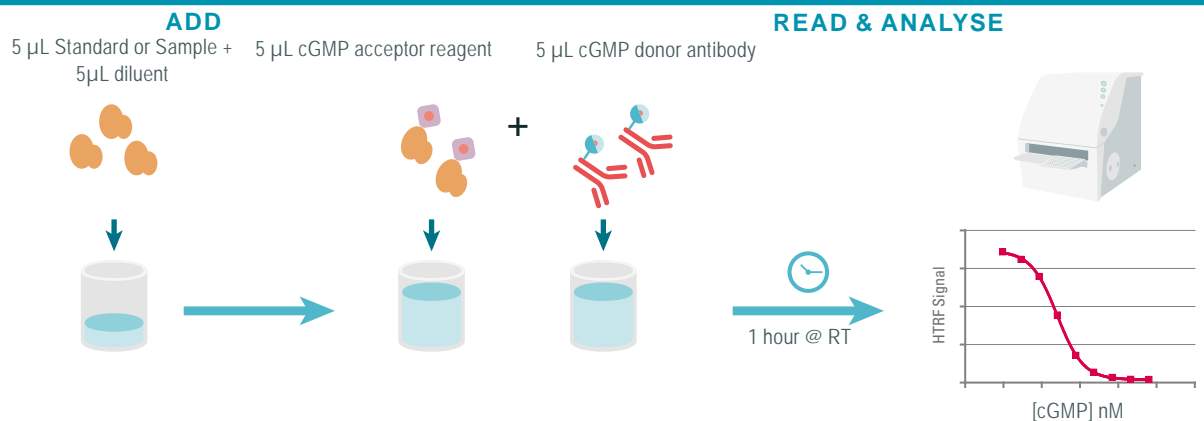


Figure 1: Principle of HTRF cGMP competitive assay.

### MANUAL AT A GLANCE



Do not pre-mix the d2 and Cryptate solutions prior to dispensing.  
 Make sure to use the set-up for Eu Cryptate.

**MATERIALS PROVIDED:**

<b>KIT COMPONENTS</b>	<b>500 TESTS * CAT # 62GM2PEG</b>	<b>10,000 TESTS * CAT # 62GM2PEH</b>
cGMP Standard Lyophilized	1 vial Concentrated free cGMP	1 vial Concentrated free cGMP
anti cGMP antibody Eu Cryptate antibody	1 vial Lyophilized	1 vial Lyophilized
cGMP d2 reagent	1 vial Lyophilized	1 vial Lyophilized
Diluent ** ready-to-use	1 vial 20 mL	1 vial 20 mL
Lysis/Detection buffer *** ready to use	1 vial 7 mL	1 vial 105 mL

\* When used as advised, the two available kit sizes will provide sufficient reagents for 500 tests and 10,000 tests respectively in 20 µL final volume..

Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.

\*\* Medium like cell culture medium can be an alternative to the diluent.

\*\*\* The Lysis/Detection buffer is used to prepare working solutions of acceptor and donor reagents.

**PURCHASE SEPARATELY:**

- HTRF®-Certified Reader. **Make sure the setup for Eu Cryptate is used.**

For a list of HTRF-compatible readers and set-up recommendations, please visit [www.revvy.com](http://www.revvy.com)

- Small volume (SV) detection microplates - .

For more information about microplate recommendations, please visit our website at: [www.revvy.com](http://www.revvy.com)

**STORAGE AND STABILITY**

Store the kit at 2-8°C.

Under proper storage conditions, reagents are stable until the expiry date indicated on the label.






If lyophilized, reconstituted reagents, antibodies, and standard stock solutions may be frozen and thawed only once. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -20°C or below (Can be stored 7 days at 4°C) .

**REAGENT PREPARATION****BEFORE YOU BEGIN:**

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Before use, allow Diluent and Lysis/Detection buffer to warm up at room temperature and homogenize them with a vortex.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- cGMP standards (for standard curve) must be prepared in diluent or in the same medium as the samples.

**TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.**


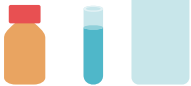


## TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 62GM2PEG			10,000 TESTS KIT - 62GM2PEH
anti cGMP antibody Eu Cryptate antibody			
<p>Reconstitute the anti cGMP antibody Eu Cryptate antibody with 2.5 mL lysis/detection buffer. Mix gently. This ready to use 1X stock solution can be frozen and stored at -20°C or below. It can be stored unfrozen 7 days at 4°C.</p> <p>When reconstituted, an orange colour, can be observed in the vial. However, this colour doesn't affect the kit performances. To avoid the apparition of this coloration, after reconstitution, the vial must be held vertically. The anti-cGMP cryptate antibody working solution (prepared with frozen stock solution) should be filtered before use to improve assay reproducibility.</p>			<p>Reconstitute the anti cGMP antibody Eu Cryptate antibody with 2.5 mL distilled water. Mix gently. This 20X stock solution can be frozen and stored at -20°C or below. It can be stored unfrozen 7 days at 4°C.</p> <p>When reconstituted, an orange colour, can be observed in the vial. However, this colour doesn't affect the kit performances. To avoid the apparition of this coloration, after reconstitution, the vial must be held vertically. The anti-cGMP cryptate antibody working solution (prepared with frozen stock solution) should be filtered before use to improve assay reproducibility.</p>
cGMP d2 reagent			
<p>Reconstitute the cGMP d2 reagent with 2.5 mL lysis/detection buffer. Mix gently. This ready to use 1X stock solution can be frozen and stored at -20°C or below. It can be stored unfrozen 7 days at 4°C.</p>			<p>Reconstitute the cGMP d2 reagent with 2.5 mL distilled water. Mix gently. This 20X stock solution can be frozen and stored at -20°C or below. It can be stored unfrozen 7 days at 4°C.</p>
cGMP Standard			
<p>Reconstitute the cGMP Standard with distilled water in order to obtain a 10,000 nM stock solution. See instructions on vial label for reconstitution volume. Mix gently after reconstitution. This stock solution can be stored 7 days at 4°C or may be frozen and stored at -20°C or below and thawed once only.</p>			<p>Reconstitute the cGMP Standard with distilled water in order to obtain a 10,000 nM stock solution. See instructions on vial label for reconstitution volume. Mix gently after reconstitution. This stock solution can be stored 7 days at 4°C or may be frozen and stored at -20°C or below and thawed once only.</p>
Diluent			
The diluent is ready-to-use			The diluent is ready-to-use
Lysis/Detection buffer			
The Lysis/Detection buffer is ready-to-use.			The Lysis/Detection buffer is ready-to-use.w

## TO PREPARE ANTIBODY WORKING SOLUTIONS:

Each well requires 5 µL anti cGMP antibody Eu Cryptate antibody and 5 µL cGMP d2 reagent.

Prepare the two solutions in separate vials.

500 TESTS KIT - 62GM2PEG			10,000 TESTS KIT - 62GM2PEH
anti cGMP antibody Eu Cryptate antibody			
<p>After reconstitution, the cGMP Eu Cryptate antibody is ready to use.</p>		<p>1 vol</p> <p>19 vol</p> 	<p>Dilute 20-fold the stock solution of cGMP Eu Cryptate antibody with Lysis/detection buffer#2 (e.g. for 10,000 tests: 2.5 mL of reconstituted reagent + 47.5 mL of Lysis/Detection buffer # 2). Mix gently. This working solution can be stored 7 days at 2-8°C or may be frozen and stored at -16°C or below and thawed once only.</p>
cGMP d2 reagent			
<p>After reconstitution, the cGMP d2 reagent is ready to use.</p>		<p>1 vol</p> <p>19 vol</p> 	<p>Dilute 20-fold the stock solution of cGMP d2 reagent with Lysis/detection buffer#2 (e.g. for 10,000 tests: 2.5 mL of reconstituted d2 reagent + 47.5 mL of Lysis/Detection buffer # 2). Mix gently. This working solution can be stored 7 days at 4°C or may be frozen and stored at -20°C or below and thawed once only.</p>
Antibody mix			
<p>Do not pre-mix the d2 and the Eu Cryptate solutions prior to dispensing.</p>			

## TO PREPARE STANDARD WORKING SOLUTIONS:

- Each well requires 5  $\mu\text{L}$  of standard.
- Dilute the standard stock solution serially with diluent
- In order to check for a potential interference effect from your own assay buffer when using the assay for the first time, we highly recommend the parallel preparation of a standard curve in your own supplemented cell culture medium and in diluent .
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

A recommended standard dilution procedure is listed and illustrated below:

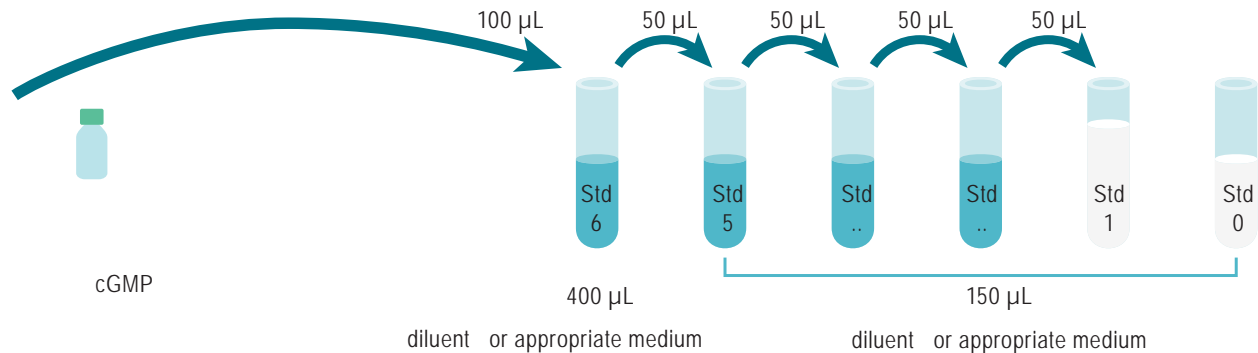
Reconstitute the cGMP standard according to the instructions on the vial label.

Dilute the standard stock solution 5-fold with diluent to prepare high standard (Std 6): e.g. take 100  $\mu\text{L}$  of standard stock solution and add it to 400  $\mu\text{L}$  of diluent . Mix gently.

Use the high standard (Std 6) to prepare the standard curve using 1/4 serial dilutions as follows:

- Dispense 150  $\mu\text{L}$  of diluent in each vial from Std 5 to Std 0.
- Add 50  $\mu\text{L}$  of standard to 150  $\mu\text{L}$  of diluent , mix gently and repeat the 1/4 serial dilution to make standard solutions: std6, std5, std4, std3, std2, std1.

This will create 6 standards for the analyte. Std 0 (Positive control) is diluent or appropriate culture medium alone.








STANDARD	SERIAL DILUTIONS	cGMP WORKING CONCENTRATION (nM)	cGMP FINAL CONC. IN ASSAY (nM)
Standard Stock solution	Reconstituted lyophilisate	10,000	
Standard 6	100 $\mu\text{l}$ reconstituted standard stock solution + 400 $\mu\text{l}$ Diluent	2,000	500
Standard 5	50 $\mu\text{L}$ standard 6 + 150 $\mu\text{L}$ Diluent	500	125
Standard 4	50 $\mu\text{L}$ standard 5 + 150 $\mu\text{L}$ Diluent	125	31.25
Standard 3	50 $\mu\text{L}$ standard 4 + 150 $\mu\text{L}$ Diluent	31.25	7.81
Standard 2	50 $\mu\text{L}$ standard 3 + 150 $\mu\text{L}$ Diluent	7.81	1.95
Standard 1	50 $\mu\text{L}$ standard 2 + 150 $\mu\text{L}$ Diluent	1.95	0.49
Standard 0	150 $\mu\text{l}$ Diluent	0	0

## TO PREPARE SAMPLES:

- Each well requires 5  $\mu$ L of sample.
- Just after their collection, put the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at -60°C or below. Avoid multiple freeze/thaw cycles.
- Samples with a concentration above the highest standard (Std 6) must be diluted diluent or in your appropriate sample medium.

## ASSAY MANUAL

		Negative control or Cryptate control	Standard (Std 0 - Std 6)	Samples
<b>Step 1</b> 	Dispense 5 $\mu$ L of diluent into each negative control well  Dispense 5 $\mu$ L diluent	Dispense 5 $\mu$ L of each cGMP standard (Std 0 - Std 6) into each standard well  Dispense 5 $\mu$ L diluent	Dispense 5 $\mu$ L of each sample into each sample well  Dispense 5 $\mu$ L diluent	
<b>Step 2</b> 	Add 5 $\mu$ L of Lysis/Detection buffer to all negative control wells	Add 5 $\mu$ L cGMP acceptor reagent working solution to all wells		
<b>Step 3</b> 	Add 5 $\mu$ L cGMP donor antibody working solution to all wells			
<b>Step 4</b> 	Seal the plate and incubate 1 hour @ RT			
<b>Step 5</b> 	Remove the plate sealer and read on an HTRF® compatible reader			



## DATA REDUCTION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

3. Calculate the % delta F which reflects the signal to background of the assay. The negative control plays the role of an internal assay control. Delta F is used for the comparison of day to day runs of the same assay.

$$\text{delta F (\%)} = \frac{\text{Ratio Standard or sample} - \text{Ratio Negative Control}}{\text{Ratio Negative Control}} \times 100$$

For more information about data reduction, please visit [www.revivity.com](http://www.revivity.com)

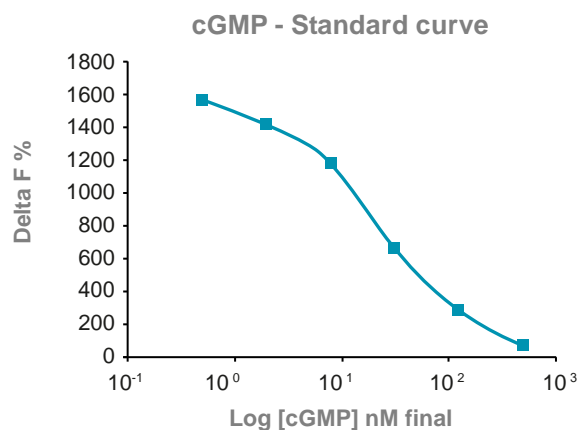
## RESULTS

This data must not be substituted for the data obtained in the laboratory and should be considered only as an example.

Results may vary from one HTRF® compatible reader to another.

The assay standard curve is created by plotting delta F% versus the analyte concentration. Delta F obtained for samples can be reported on the standard curve to deduce respective cGMP concentration.

	Ratio <sup>(1)</sup>	CV <sup>(2)</sup>	Delta F% <sup>(3)</sup>
Negative control	393	1%	
Std 0 - Positive control	6,887	3.6%	1,654%
Std 1 - 0.49 nM final	6,423	1.1%	1,536%
Std 2 - 1.95 nM final	5,967	0.9%	1,42%
Std 3 - 7.81 nM final	4,569	0.7%	1,064%
Std 4 - 31.25 nM final	2,855	2.6%	627%
Std 5 - 125 nM final	1,413	0.3%	260%
Std 6 - 500 nM final	717	1%	83%



## CELL-BASED ASSAY

Cell-based assay manual includes 2 main steps :

1. Cell and compound dispensing, followed by incubation
2. Detection with HTRF reagents diluted in Lysis/detection buffer #1 (500 tests) and Lysis/detection buffer #2 (10,000 tests)

The cell density optimization is a key step in cyclic GMP kit use. Typically, the level of cGMP produced by cells must fall within the linear range of the standard curve.

### PLEASE FIND BELOW SOME ADVICES FOR OPTIMIZATION PRIOR USING REVVITY KIT:

The optimization consists of testing a wide range of cell concentrations (e.g. between 300 and 20.000 cells per well), in the presence or absence of a direct activator of the cell Guanylate Cyclase (GC) enzyme, such as NO donor S-nitroso-N-acetylpenicil amine (SNAP) for soluble GC or Atrial Natriuretic Peptide (ANP) for the particulate GC. The addition of a phosphodiesterase inhibitor in cell dilution buffer is absolutely necessary (e.g. IBMX) in order to prevent cGMP degradation. The optimum cell density is the number of cells per well which leads to the highest signal amplitude obtained between the inactivated state (basal level of cGMP produced by cells) and the activated condition.

### CELL-BASED ASSAY MANUAL

Cell negative control	Cell condition
5 µL cells	5 µL cells
5 µL compound buffer	5 µL test compound
Cell stimulation (e.g. 30 minutes)	Cell stimulation (e.g. 30 minutes)
5 µL Lysis/detection buffer #1/#2	5 µL cGMP-d2
5 µL anti cGMP-Cryptate antibody	5 µL anti cGMP-Cryptate antibody

Seal the plate and leave to incubate at room temperature for 1 hour.  
Remove the plate sealer and read on an HTRF® compatible reader.

## ANALYTICAL CHARACTERISTICS

### SPECIFICITY

Analyte	% cross-reactivity
cGMP	100
GMP	0.003
GDP	0.001
GTP	0.003
cAMP	<0.001
AMP	<0.001
ATP	<0.001

### DETECTION LIMIT

	cGMP working concentration (nM)	cGMP final con. in assay (nM)
Detection limit	2.8	0.7
EC50	84	21

REACH European regulations and compliance. This product and/or some of its components include a Triton concentration of 0.1% or more and as such, it is concerned by the REACH European regulations. We recommend researchers using this product to act in compliance with REACH and in particular: to only use the product for in vitro research in appropriate and controlled premises by qualified researchers, ii) to ensure the collection and the treatment of subsequent waste, and iii) to make sure that the total amount of Triton handled does not exceed 1 ton per year.

This product contains material of biologic origin. Use for research purposes only. Do not use in humans or for diagnostic purposes. The purchaser assumes all risk and responsibility concerning reception, handling and storage. The use of the cell line will be done with appropriate safety and handling precautions to minimize health and environmental impact. Remaining disclaimer.





The information provided in this document is for reference purposes only and may not be all-inclusive. Revvity, Inc., its subsidiaries, and/or affiliates (collectively, "Revvity") do not assume liability for the accuracy or completeness of the information contained herein. Users should exercise caution when handling materials as they may present unknown hazards. Revvity shall not be liable for any damages or losses resulting from handling or contact with the product, as Revvity cannot control actual methods, volumes, or conditions of use. Users are responsible for ensuring the product's suitability for their specific application. REVVITY EXPRESSLY DISCLAIMS ALL WARRANTIES, INCLUDING WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, REGARDLESS OF WHETHER ORAL OR WRITTEN, EXPRESS OR IMPLIED, ALLEGEDLY ARISING FROM ANY USAGE OF ANY TRADE OR ANY COURSE OF DEALING, IN CONNECTION WITH THE USE OF INFORMATION CONTAINED HEREIN OR THE PRODUCT ITSELF

Manufactured by Cisbio Bioassays - Parc Marcel Boiteux - 30200 Codolet - FRANCE

[www.revvity.com](http://www.revvity.com)

revvity

**Revvity, Inc.**  
940 Winter Street  
Waltham, MA 02451 USA  
[www.revvity.com](http://www.revvity.com)

For a complete listing of our global offices, visit [www.revvity.com](http://www.revvity.com)  
Copyright ©2023, Revvity, Inc. All rights reserved.