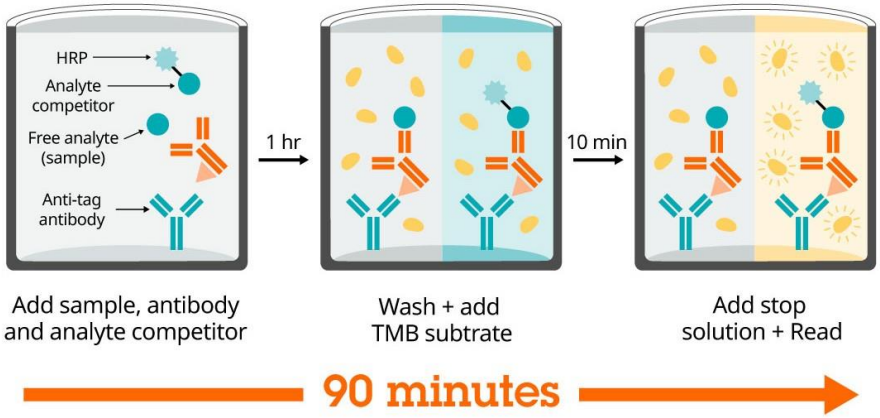
# ab323639 – cGMP SimpleStep ELISA® Kit – Intracellular (Cyclic GMP)

For the quantitative measurement of cGMP in cell and tissue extracts.
For research use only - not intended for diagnostic use.

**For overview, typical data and additional information please visit: www.abcam.com/ab323639**




**Storage and Stability:** Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

## Materials Supplied

| Item | Quantity 1 x 96 tests | Quantity 10 x 96 tests | Storage Condition |
|---|---|---|---|
| cGMP CaptSure™ Conjugate 50X | 80 µL | 10 x 80 µL | +4°C |
| cGMP HRP Conjugate 50X | 80 µL | 10 x 80 µL | +4°C |
| cGMP Lyophilized Standard | 2 Vials | 10 x 2 Vials | +4°C |
| Antibody Diluent 4BI | 8 mL | 10 x 8 mL | +4°C |
| Cell Extraction Buffer PTR 5X | 10 mL | 2 x 50 mL | +4°C |
| Sample Diluent NS | 12 mL | - | +4°C |
| Wash Buffer PT 10X | 20 mL | 200 mL | +4°C |
| TMB Development Solution | 12 mL | 120 mL | +4°C |
| Stop Solution | 12 mL | 120 mL | +4°C |
| SimpleStep Pre-Coated 96-Well Microplate | 96 wells | 10 x 96 wells | +4°C |
| Plate Seal | 1 | 10 | +4°C |

Sample Diluent NS is provided but not necessary for this product.

## Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Microplate reader capable of measuring absorbance at 450 or 600 nm.
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Method for determining total protein concentration in sample (BCA assay recommended).
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

## Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. The CaptSure and HRP Conjugates have only been tested for stability in the provided 50X formulation.

**1X Cell Extraction Buffer PTR (For cell and tissue extracts only):** Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 8 mL deionized water and 2 mL Cell Extraction Buffer PTR 5X. Mix thoroughly and gently. If required protease inhibitors can be added.

**1X Wash Buffer PT:** Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

**CaptSure Conjugate Solution:** Prepare CaptSure Conjugate Solution by diluting the 50X CaptSure Conjugate in Antibody Diluent 4BI. To make 2 mL of the Capture Conjugate Solution combine 40 µL 50X CaptSure Conjugate with 1.96 mL Antibody Diluent 4BI. Mix thoroughly and gently.

**HRP Conjugate Solution:** Prepare HRP Conjugate Solution by diluting the 50X HRP Conjugate in Antibody Diluent 4BI. To make 2 mL of the HRP Conjugate Solution combine 40 µL 50X HRP Conjugate with 1.96 mL Antibody Diluent 4BI. Mix thoroughly and gently.

## Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

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1. Reconstitute the cGMP standard sample by adding the volume of 1X Cell Extraction Buffer PTR indicated on the standard vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 50,000 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 288 µL of 1X Cell Extraction Buffer PTR into tube number 1 and 180 µL of 1X Cell Extraction Buffer PTR into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no standard and is the Blank control:

| Standard # | Dilution Sample | Volume to Dilute (µL) | Volume of Diluent (µL) | Starting Conc. (pg/mL) | Final Conc. (pg/mL) |
|---|---|---|---|---|---|
| 1 | **Stock Standard** | 72 | 288 | 50,000 | 10,000 |
| 2 | Standard#1 | 180 | 180 | 10,000 | 5,000 |
| 3 | Standard#2 | 180 | 180 | 5,000 | 2,500 |
| 4 | Standard#3 | 180 | 180 | 2,500 | 1,250 |
| 5 | Standard#4 | 180 | 180 | 1,250 | 625 |
| 6 | Standard#5 | 180 | 180 | 625 | 312.5 |
| 7 | Standard#6 | 180 | 180 | 312.5 | 156.3 |
| 8 | Blank Control | 0 | 180 | N/A | N/A |

## Sample Preparation

| Typical Sample Dynamic Range | |
|---|---|
| **Sample Type** | **Range** |
| HepG2 Cell Extract | 0.063 - 0.5 mg/mL |
| Human Kidney Tissue Extract | 0.063 - 0.5 mg/mL |

**Preparation of extracts from cell pellets** Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C. Rinse cells twice with PBS. Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract maybe quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

**Preparation of extracts from adherent cells by direct lysis (alternative protocol)** Remove growth media and rinse adherent cells 2 times in PBS. Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate). Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

**Preparation of extracts from tissue homogenates** Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended). Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

## Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
Differences in well absorbance or "edge effects" have not been observed with this assay.

## Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 60 µL of all sample or standard to appropriate wells.
4. Add 30 µL of the CaptSure Conjugate Solution to each well.
    a. Optional – for non-specific binding wells, add 30 µL of Antibody Diluent 4BI in lieu of CaptSure Conjugate Solution.
5. Add 30 µL of the HRP Conjugate Solution to each well.
6. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
7. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 30 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
8. Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
   *Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.*
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
9. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
10. Alternative to 8 – 9: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

| Mode | Kinetic |
|---|---|
| Wavelength: | 600 nm |
| Time: | up to 20 min |
| Interval: | 20 sec - 1 min |
| Shaking: | Shake between readings |

**Note** that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

**Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:**
www.abcam.com/protocols/the-complete-elisa-guide

**For technical support contact information, visit:** www.abcam.com/contactus

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## Additional information

### ASSAY SPECIFICITY
This kit is designed for the quantification of cGMP.

The standard in this kit is free cGMP.

Native signal was detected in SIN-1- and IBMX-treated HepG2 cell extract.

Spiked experiments were used to validate cell and tissue extracts.

Serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, saliva, urine, milk, and CSF samples have not been tested with this kit.

For the measurement of cGMP in serum, plasma, cell culture supernatant, saliva, urine, and CSF, use cGMP ELISA Kit - Extracellular ab323638.

### CROSS REACTIVITY
Cross reactivity was determined for related compounds GMP, GDP, GTP, cAMP, AMP, ADP, GTP, CMP, CDP, CTP and cUMP at 10,000 and 100,000 pg/mL. No cross reactivity was observed.

### INTERFERENCE
10,000 and 100,000 pg/mL of GMP, GDP, GTP, cAMP, AMP, ADP, ATP, CMP, CDP, CTP and cUMP were tested for interference with 2,500 pg/mL of cGMP. No interference was observed.

### SPECIES REACTIVITY
Validated in Human samples, reactivity is species independent.

### CALCULATION
- Optional: Non-specific binding (NSB) well subtracted values can be calculated by averaging the absorbance values for the NSB wells and subtracting the average NSB absorbance value from all other absorbance values.
- Create a standard curve by plotting the average absorbance value for each standard concentration (y-axis) against the target concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
- Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target analyte in the sample by interpolating absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target analyte in the sample.
- Samples generating absorbance values lower than that of the highest concentration standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance value greater than that of the lowest concentration standard should be retested in a less dilute form.
- Optional: The binding percentage, $B/B_0$, can be calculated by dividing the average absorbance value for each standard or sample by the average absorbance of the zero standard ($B_0$).

### TYPICAL DATA
Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

| Standard Curve Measurements | | | | |
|---|---|---|---|---|
| Concentration (pg/mL) | O.D. 450 nm | | Mean O.D. | $B/B_0$ (%) |
| | 1 | 2 | | |
| NSB | 0.035 | 0.039 | 0.037 | - |
| 0 ($B_0$) | 1.739 | 1.732 | 1.735 | 100% |
| 156.3 | 1.558 | 1.551 | 1.554 | 90% |
| 312.5 | 1.413 | 1.447 | 1.430 | 82% |
| 625 | 1.173 | 1.217 | 1.195 | 69% |
| 1,250 | 0.913 | 0.924 | 0.918 | 53% |
| 2,500 | 0.581 | 0.580 | 0.580 | 33% |
| 5,000 | 0.367 | 0.368 | 0.368 | 21% |
| 10,000 | 0.227 | 0.228 | 0.228 | 13% |

Table 1. Example of cGMP standard curve in 1X Cell Extraction Buffer PTR. The cGMP standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

### TYPICAL SAMPLE VALUES
#### Sensitivity:
The calculated minimal detectable dose (MDD) is 93.7 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=24) and subtracting 2 standard deviations then extrapolating the corresponding concentration.

#### Recovery
3 concentrations of cGMP were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

| Sample Type | Average % Recovery | Range (%) |
|---|---|---|
| 0.5 mg/mL HepG2 Extract | 100 | 87 – 107 |
| 0.5 mg/mL Human Kidney Extract | 98 | 96 – 99 |

## Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native cGMP was measured in the following biological sample in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

| Dilution Factor | Interpolated value | 0.5 mg/mL SIN-1- and IBMX-treated HepG2 Cell Extract |
|---|---|---|
| Undiluted | pg/mL | 1,681 |
| | **% Expected** | 100 |
| 2 | pg/mL | 817 |
| | **% Expected** | 97 |
| 4 | pg/mL | 471 |
| | **% Expected** | 112 |
| 8 | pg/mL | 241 |
| | **% Expected** | 115 |

Free cGMP was spiked in in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

| Dilution Factor | Interpolated value | 0.5 mg/mL HepG2 Cell Extract | 0.5 mg/mL Human Kidney Extract |
|---|---|---|---|
| Undiluted | pg/mL | 2,806 | 2,867 |
| | **% Expected** | 100 | 100 |
| 2 | pg/mL | 1,529 | 1,496 |
| | **% Expected** | 109 | 104 |
| 4 | pg/mL | 737 | 766 |
| | **% Expected** | 105 | 107 |
| 8 | pg/mL | 402 | 420 |
| | **% Expected** | 115 | 117 |

## Precision

Mean coefficient of variations of interpolated values of cGMP from one concentration of SIN-1- and IBMX-treated HepG2 Cell Extract within the working range of the assay.

| | Intra-assay | Inter-assay |
|---|---|---|
| **N=** | 8 | 3 |
| **CV (%)** | 6.2 | 2.1 |

**Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:**
www.abcam.com/protocols/the-complete-elisa-guide
**For technical support contact information, visit:** www.abcam.com/contactus

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