cGAS Inhibitor Screening Assay Kit

Item No. 701930

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Materials Supplied

Kit will arrive packaged as a -80°C kit. After opening the kit, store individual components as stated below.

Item Number	Item	Quantity/Size	Storage
701931	cGAS Reaction Buffer (2X)	1 vial/6 ml	-20°C
701933	cGAS Enzyme (human, recombinant)	1 vial	-80°C
701932	cGAS Substrate	1 vial/1 ml	-20°C
701934	cGAS Inhibitor (CU-76)	1 vial/25 µl	-20°C
701935	cGAS Stop Solution	1 vial/1 ml	-20°C
701936	cGAS Inhibitor Screening ELISA Polyclonal Antiserum (1X)	1 vial/100 dtn	4°C
701937	cGAS Inhibitor Screening ELISA HRP Tracer (6X)	1 vial/100 dtn	4°C
701938	cGAS Inhibitor Screening ELISA Standard	1 vial	4°C
401703	Immunoassay Buffer C Concentrate (10X)	1 vial/10 ml	4°C
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
400035	Polysorbate 20	1 vial/3 ml	RT
400074	TMB Substrate Solution	2 vials/12 ml	4°C
10011355	HRP Stop Solution	1 vial/12 ml	RT
400004/400006	Mouse Anti-Rabbit IgG-Coated Plate	1 plate	4°C
400012	96-Well Cover Sheet	1 ea	RT

If any of the items listed on page 3 are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified.

When compared to quantification by LC/MS or GC/MS, it is not uncommon for immunoassays to report higher analyte concentrations. While LC/MS or GC/MS analyses typically measure only a single compound, antibodies used in immunoassays sometimes recognize not only the target molecule, but also structurally related molecules, including biologically relevant metabolites. In many cases, measurement of both the parent molecule and metabolites is more representative of the overall biological response than is the measurement of a short-lived parent molecule. It is the responsibility of the researcher to understand the limits of both assay systems and to interpret their data accordingly.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm
2. Adjustable pipettes; multichannel recommended
3. An orbital microplate shaker
4. A 37°C water bath, heat block, or incubator
5. Reaction tubes; PCR plates or tube strips recommended
6. A source of ultrapure water is recommended. Pure water - glass-distilled or deionized - may not be acceptable *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
7. Materials used for **cGAS Inhibitor Preparation** (see page 10)

INTRODUCTION

Background

Cyclic GMP-AMP (cGAMP) synthase (cGAS) is a nucleotidyltransferase located in the cytosol of mammalian cells that acts as a dsDNA sensor to detect foreign DNA from microbial pathogens as part of the innate immune response.[1,2] Upon binding to dsDNA, cGAS produces the cyclic dinucleotide second messenger cGAMP, which activates stimulator of interferon genes (STING), leading to activation of the type I IFN pathway.[1-3] cGAS is localized to the cytosol and, in cases of nuclear envelope disruption, is outcompeted for dsDNA binding by the nuclear chromatin-binding protein BAF to restrict cGAS activation by self, genomic dsDNA.[4] Activation of cGAS by pathogen-associated dsDNA and the production of 2'3'-cGAMP are important in host defense but may play a role in the development of autoimmune diseases, such as systemic lupus erythematosus (SLE), which are characterized by increased expression of IFN-stimulated genes.[5] Additionally, cGAS is activated in response to mitochondrial DNA leakage, which is associated with metastatic phenotypes and age-associated inflammation in cancer, and the accumulation of extrachromosomal telomere repeat DNA that results in IFN expression and inhibition of cell proliferation.[5,6] Late stage tumors with a high level of chromosomal instability exhibit decreased protein expression of cGAS as a mechanism to evade cGAS-mediated IFN-signaling and inhibition of tumor growth.[7] In contrast, carcinogen-induced cGAS activation and transfer of tumor cell cGAS to astrocytes through gap junctions promotes tumorigenesis and brain metastasis, respectively, in mouse models. Inhibition of cGAS activity suppresses IFN-stimulated gene expression and decreases type I IFN production in patient-derived samples and mouse models of autoimmune diseases, indicating therapeutic utility of cGAS inhibition.

About This Assay

Cayman's cGAS Inhibitor Screening Assay Kit provides a robust and easy-to-use platform for identifying novel inhibitors of human cGAS, which is a cytosolic dsDNA sensor that detects microbial pathogens. The assay directly measures 2'3'-cGAMP produced by cGAS in the presence of DNA, ATP, and GTP. The cyclic dinucleotide product of that reaction is quantified *via* ELISA using a 2'3'-cGAMP-specific antiserum. The cGAS inhibitor CU-76 is included as a positive control.

The ELISA portion of Cayman's cGAS Inhibitor Screening Assay Kit is a competitive assay with a range of 4.57-10,000 pM, a midpoint of approximately 300 pM (50% B/B_0), and a sensitivity (80% B/B_0) of approximately 50 pM.

Principle of the ELISA

The readout for the cGAS enzymatic reaction uses an ELISA based on the competition between 2'3'-cGAMP produced in the cGAS reaction and a 2'3'-cGAMP-HRP conjugate, cGAS Inhibitor Screening ELISA HRP Tracer (HRP tracer), for a limited amount of 2'3'-cGAMP polyclonal antiserum, cGAS Inhibitor Screening ELISA Polyclonal Antiserum (polyclonal antiserum). Because the concentration of the HRP tracer is held constant while the concentration of 2'3'-cGAMP produced from the cGAS reaction varies, the amount of HRP tracer that is able to bind to the polyclonal antiserum will be inversely proportional to the concentration of 2'3'-cGAMP produced from the cGAS reaction. This antibody-2'3'-cGAMP complex binds to mouse anti-rabbit IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and TMB Substrate Solution (which contains the substrate to HRP) is added to the well, followed by the HRP Stop Solution. The final product of this reaction has a distinct yellow color that can be measured at 450 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of cGAS Inhibitor Screening ELISA HRP Tracer bound to the well, which is inversely proportional to the amount of 2'3'-cGAMP produced from the cGAS reaction in the well during the incubation, as described in the equation:

$$\text{Absorbance} \propto [\text{Bound 2'3'-cGAMP-HRP Tracer}] \propto 1/[\text{2'3'-cGAMP produced from the cGAS reaction}]$$

A schematic of this process is shown in Figure 1, on page 9.

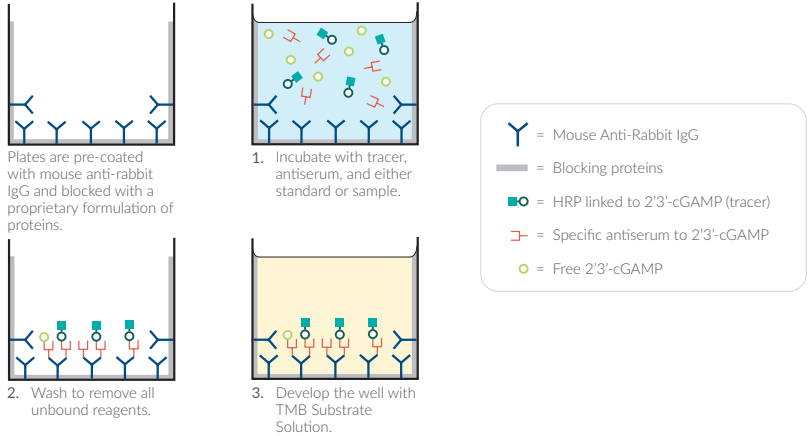


Figure 1. Schematic of the cGAS Inhibitor Screening ELISA

cGAS REACTION PROCEDURE

The ELISA plate will allow for 36 cGAS reactions (in duplicate) at one dilution or 18 cGAS reactions (in duplicate) at two dilutions.

IMPORTANT: Please read both the cGAS Reaction Procedure and ELISA Procedure sections carefully before initiating experiments!

cGAS Inhibitor Preparation

This assay is sensitive to solvent concentrations. Unknown inhibitors can be dissolved in ultrapure water, PBS, dimethyl formamide (DMF), DMSO, ethanol, or methanol. To determine an IC_{50} value for an inhibitor, multiple concentrations of the inhibitor should be tested in the assay. A final volume of 5 µl of inhibitor should be added to each unknown inhibitor reaction tube. Table 1 shows solvent tolerance with a full solvent titration shown in Figure 6 on page 36. If solvent concentrations are outside of the values in Table 1, it is important that 100% initial activity reactions contain a final solvent concentration that corresponds to the matching inhibitor reaction. Remember to account for the 1:40 dilution (5 µl of unknown inhibitor into 200 µl final reaction volume) when preparing unknown inhibitor stock solutions.

Solvent	Tolerance
DMF	0.1%
DMSO	2%
Ethanol	0.1%
Methanol	2%

Table 1. cGAS Reaction Solvent Tolerance

cGAS Reagent Preparation

1. **cGAS Reaction Buffer (1X)**

   Dilute 0.5 ml of cGAS Reaction Buffer (2X) (Item No. 701931) with 0.5 ml of ultrapure water to make 1 ml of cGAS Reaction Buffer (1X), which will be used to dilute the supplied cGAS enzyme. The remaining cGAS Reaction Buffer (2X) will be used in the cGAS reaction. The cGAS Reaction Buffer (1X) may be stored at -20°C where it will be stable for at least six months.

2. **cGAS Substrate**

   The cGAS Substrate (Item No. 701932) included with this kit is ready to use as supplied. If all of the cGAS Substrate will not be used at one time, aliquot the undiluted substrate and store at -20°C where it will be stable for at least six months.

3. **cGAS Enzyme (human, recombinant)**

   cGAS Enzyme (human, recombinant) (Item No. 701933) should be thawed on ice and mixed prior to dilution. To dilute the enzyme, mix 80 µl of cGAS Enzyme (human, recombinant) with 0.72 ml of cGAS Reaction Buffer (1X). This volume of diluted cGAS enzyme is sufficient for 40 reactions. It is recommended that the enzyme be diluted immediately prior to performing the assay. The diluted enzyme loses 30% of its activity when stored on ice for one hour. If performing fewer than 36 reactions, the undiluted enzyme can be aliquoted and stored at -80°C, limiting freeze-thaw cycles.

4. **cGAS Inhibitor (CU-76)**

   This vial contains 25 µl of 100 µM cGAS Inhibitor (CU-76) (Item No. 701934) in DMSO, which can be used as a positive control. This positive control is ready to use as supplied. If all of the cGAS Inhibitor (CU-76) will not be used at one time, aliquot the undiluted inhibitor and store at -20°C where it will be stable for at least six months.

5. **cGAS Stop Solution**

   The cGAS Stop Solution (Item No. 701935) included with this kit is ready to use as supplied. Store any unused cGAS Stop Solution at room temperature where it will be stable for at least one year.

General Information

- The final volume of the assay is 200 µl in all the reaction tubes.
- Use cGAS Reaction Buffer (2X) in the assay. cGAS Reaction Buffer (1X) is only used to dilute the cGAS enzyme.
- Both the concentrated and diluted cGAS enzyme should be kept on ice until ready to initiate the reaction.
- All other reagents should be prepared as described in the cGAS Reagent Preparation section and kept at room temperature before beginning the assay.
- The ELISA plate will allow for 36 cGAS reactions (in duplicate) at one dilution or 18 cGAS reactions (in duplicate) at two dilutions.
- The assay temperature is 37°C. Set the water bath, heat block, or incubator temperature to 37°C before initiating the reactions.

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the reaction tubes. This saves time and helps maintain more precise incubation times.
- Use different tips to pipette the buffer, substrate, enzyme, inhibitor, and stop solution.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the reaction tube.

Performing cGAS Reactions

1. **Ultrapure Water:** add 65 µl of ultrapure water to all reaction tubes.
2. **Solvent:** add 5 µl of solvent corresponding to that used in inhibitor preparation to 100% initial activity tubes. *NOTE: if inhibitors in different solvents are to be assayed at the same time, separate sets of 100% initial activity reactions should be run for each solvent and/or concentration.*
3. **Inhibitor/Positive Control:** add 5 µl of the unknown inhibitor or the 100 µM positive control, cGAS Inhibitor (CU-76), to the respective reaction tubes.
4. **cGAS Reaction Buffer:** add 90 µl of cGAS Reaction Buffer (2X) to all reaction tubes.
5. **cGAS Substrate:** add 20 µl of cGAS Substrate to all reaction tubes.
6. **cGAS Enzyme (human, recombinant):** initiate the reactions by adding 20 µl of diluted cGAS Enzyme (human, recombinant) to all reaction tubes.
7. Incubate for 30 minutes at 37°C.
8. **cGAS Stop Solution:** add 20 µl of cGAS Stop Solution to all reaction tubes and mix well.
7. The 2'3'-cGAMP product produced during these cGAS reactions is quantified by ELISA. Proceed to the ELISA Procedure (see page 16). Alternatively, the reaction products can be stored at -80°C and quantified at a later date.

	100% Initial Activity	Positive Control (CU-76)	Unknown Inhibitor
Ultrapure Water	65 µl		
Solvent	5 µl	--	--
Positive Control (CU-76)	--	5 µl	--
Unknown Inhibitor (40X)	--	--	5 µl
cGAS Reaction Buffer (2X)	90 µl		
cGAS Substrate	20 µl		
Diluted cGAS Enzyme (human, recombinant)	20 µl		
Incubate	Incubate for 30 minutes at 37°C		
cGAS Stop Solution	20 µl		

Table 2. cGAS Reaction Set-up Summary

ELISA PROCEDURE

Definition of Key Terms

Blank (Blk): background absorbance caused by TMB Substrate Solution and the HRP Stop Solution. The blank absorbance should be subtracted from the absorbance readings of all the other wells, including the non-specific binding (NSB) wells.

Total Activity (TA): total enzymatic activity of the cGAS inhibitor screening ELISA HRP-linked tracer.

NSB (Non-Specific Binding): non-immunological binding of the tracer to the well. Even in the absence of specific antiserum a very small amount of tracer still binds to the well; the NSB is a measure of this low binding.

B_0 (Maximum Binding): maximum amount of the tracer that the antiserum can bind in the absence of free analyte.

%B/B_0 (%Bound/Maximum Bound): ratio of the absorbance of a sample or standard well to the average absorbance of the maximum binding (B_0) wells.

Standard Curve: a plot of the %B/B_0 values *versus* concentration of a series of wells containing various known amounts of analyte.

Dtn: determination, where one dtn is the amount of reagent used per well.

Cross Reactivity: numerical representation of the relative reactivity of this assay towards structurally related molecules as compared to the primary analyte of interest. Biomolecules that possess similar epitopes to the analyte can compete with the assay tracer for binding to the antiserum. Substances that are superior to the analyte in displacing the tracer result in a cross reactivity that is greater than 100%. Substances that are inferior to the primary analyte in displacing the tracer result in a cross reactivity that is less than 100%. Cross reactivity is calculated by comparing the mid-point (50% B/B_0) value of the tested molecule to the mid-point (50% B/B_0) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\%\ \text{Cross Reactivity} = \left[\frac{50\%\ B/B_0 \text{ value for the primary analyte}}{50\%\ B/B_0 \text{ value for the potential cross reactant}} \right] \times 100\%$$

Lower Limit of Detection (LLOD): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

ELISA Buffer Preparation

Store all diluted buffers at 4°C; they should be stable for approximately two months. NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water.

1. **Immunoassay Buffer C (1X) Preparation**

   Dilute the contents of one vial of Immunoassay Buffer C Concentrate (10X) (Item No. 401703) with 90 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated.

2. **Wash Buffer (1X) Preparation**

   Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with ultrapure water to a total volume of 2 L and add 1 ml of Polysorbate 20 (Item No. 400035). Smaller volumes of Wash Buffer (1X) can be prepared by diluting the Wash Buffer Concentrate (400X) 1:400 and adding Polysorbate 20 to an end concentration of 0.5 ml/L. *NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.*

ELISA Sample Preparation

To fall within the usable range of the ELISA, all cGAS reaction products should be diluted 1:5 with Immunoassay Buffer C (1X). If running multiple dilutions of the same reaction, an additional 1:2 dilution (1:10 final) is recommended. For each dilution, a minimum volume of 125 µl is required to run the ELISA.

ELISA Reagent Preparation

cGAS Inhibitor Screening ELISA Standard

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-8. Aliquot 450 µl Immunoassay Buffer C (1X) to tube #1 and 400 µl Immunoassay Buffer C (1X) to tubes #2-8. Transfer 50 µl of the cGAS Inhibitor Screening ELISA Standard (Item No. 701938) to tube #1 and mix thoroughly. Serially dilute the standard by removing 200 µl from tube #1 and placing it in tube #2; mix thoroughly. Next, remove 200 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8.

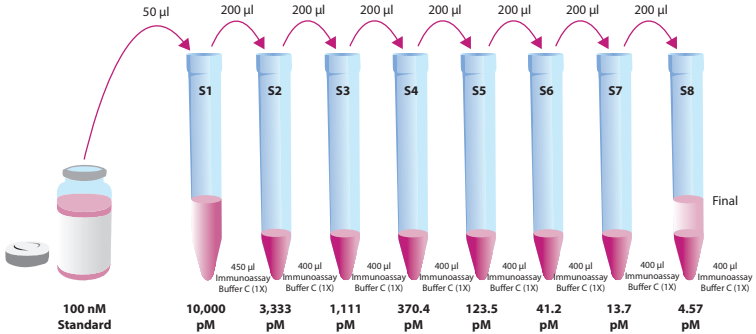


Figure 2. Preparation of the cGAS Inhibitor Screening ELISA standards

cGAS Inhibitor Screening ELISA HRP Tracer (1X)

Dilute the cGAS Inhibitor Screening ELISA HRP Tracer (6X) (Item No. 701937) with 5 ml of Immunoassay Buffer C (1X). Store the cGAS Inhibitor Screening ELISA HRP Tracer (1X) at 4°C (*do not freeze the 1X tracer!*) and use within four weeks. A 20% surplus of tracer has been included to account for any incidental losses.

cGAS Inhibitor Screening ELISA Polyclonal Antiserum (1X)

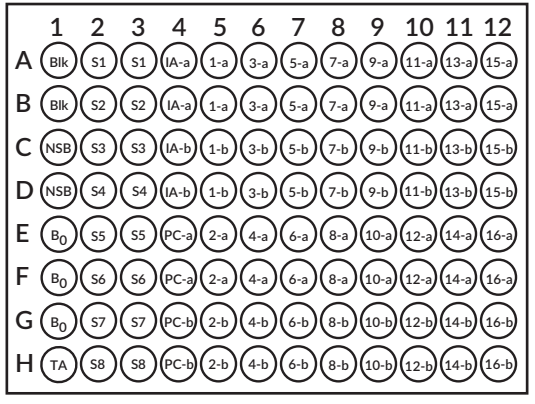
The cGAS Inhibitor Screening ELISA Polyclonal Antiserum (1X) (Item No. 701936) is ready to use as supplied. After thawing, store the cGAS Inhibitor Screening ELISA Polyclonal Antiserum at 4°C (*do not refreeze!*) and use within six months. A 20% surplus of antiserum has been included to account for any incidental losses.

Plate Set Up

The 96-well plate(s) included with this kit must be pre-washed five times with Wash Buffer (1X) (~300 µl/well) prior to use in the ELISA. *NOTE: Do not store strips after pre-washing. If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two Blk, two NSB, and three B_0 wells, and an eight-point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each cGAS reaction product is recommended to be assayed at two dilutions and each dilution should be assayed in duplicate in the ELISA.

A suggested plate format is shown in Figure 3, on page 21. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 25 for more details). We suggest you record the contents of each well on the template sheet provided (see page 41).



Blk - Blank
NSB - Non-Specific Binding
TA - Total Activity
B_0 - Maximum Binding
S1-S8 - Standards 1-8
IA-(a/b) 100% Initial Activity (dilution a/b)
PC-(a/b) Positive Control (dilution a/b)
1-16-(a/b) Reaction Products (dilution a/b)

Figure 3. Sample plate format

Performing the ELISA

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Plate Preparation

Rinse the plate (or strips to be used) five times with ~300 µl Wash Buffer (1X).

Addition of the Reagents

1. **Immunoassay Buffer C**

   Add 100 µl Immunoassay Buffer C (1X) to NSB wells. Add 50 µl Immunoassay Buffer C (1X) to B_0 wells.

2. **cGAS Inhibitor Screening ELISA Standard**

   Add 50 µl from tube #8 to both of the lowest standard wells (S8). Add 50 µl from tube #7 to each of the next standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

3. **cGAS Reaction Products**

   Add 50 µl diluted cGAS reaction product per well. Two dilutions are recommended for each cGAS reaction product. Each dilution should be assayed in duplicate.

4. **cGAS Inhibitor Screening ELISA HRP Tracer**

   Add 50 µl to each well *except* the TA and Blk wells.

5. **cGAS Inhibitor Screening ELISA Polyclonal Antiserum**

   Add 50 µl to each well, *except* the TA, NSB, and Blk wells, within 15 minutes of addition of the tracer.

Incubation of the Plate

Cover each plate with a 96-Well Cover Sheet (Item No. 400012) and incubate two hours at room temperature on an orbital shaker.

Development of the Plate

1. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X).
2. Add 175 µl of TMB Substrate Solution (Item No. 400074) to each well.
3. Dilute 10 µl cGAS Inhibitor Screening ELISA HRP Tracer (1X) with 40 µl of Immunoassay Buffer C (1X). Add 5 µl of the diluted tracer to the TA wells.
4. Cover the plate with the 96-well Cover Sheet. Optimum development is obtained by using an orbital shaker at room temperature for 30 minutes.
5. Remove the plate cover being careful to keep TMB Substrate Solution from splashing on the cover. *NOTE: Any loss of TMB Substrate Solution will affect the absorbance readings.*
6. **DO NOT WASH THE PLATE**. Add 75 µl of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The stop solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Read the plate at a wavelength of 450 nm.

Procedure	Blk	TA	NSB	B_0	Standards/ Samples
Plate Preparation	Wash strips to be used for the assay 5 x ~ 300 µl Wash Buffer (1X)				
Dilute and mix	Mix all reagents gently				
Immunoassay Buffer C (1X)	--	--	100 µl	50 µl	--
Standards/Samples	--	--	--	--	50 µl
HRP Tracer	--	--	50 µl	50 µl	50 µl
Polyclonal Antiserum	--	--	--	50 µl	50 µl
Seal	Seal the plate				
Incubate	Incubate plate 2 hours at room temperature on an orbital shaker				
Aspirate and Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)				
TMB Substrate Solution	Apply 175 µl TMB Substrate Solution				
Diluted Tracer	--	5 µl	--	--	--
Seal	Seal plate and incubate for 30 minutes at room temperature on an orbital shaker protected from light				
HRP Stop Solution	Apply 75 µl HRP Stop Solution				
Read	Read optical density at 450 nm				

Table 3. cGAS Inhibitor Screening ELISA Set-up Summary

ELISA ANALYSIS

Many plate readers come with data reduction software that plots data automatically. Alternatively, a spreadsheet program can be used. The data should be plotted as either %B/B_0 *versus* log concentration using a four-parameter logistic fit or as logit B/B_0 *versus* log concentration using a linear fit. *NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysisTools/ELISA) to obtain a free copy of this convenient data analysis tool.*

Preparation of the ELISA Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

NOTE: If the plate reader has not subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate, be sure to do that now.

1. Average the absorbance readings from the NSB wells.
2. Average the absorbance readings from the B_0 wells.
3. Subtract the NSB average from the B_0 average. This is the corrected B_0 or corrected maximum binding.
4. Calculate the B/B_0 (Sample or Standard Bound/Maximum Bound) for the remaining wells. To do this, subtract the average NSB absorbance from the S1 absorbance and divide by the corrected B_0 (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain %B/B_0 for a logistic four-parameter fit, multiply these values by 100.)

NOTE: The TA values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool. Low or no absorbance from a TA well could indicate a dysfunction in the enzyme-substrate system.

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 *versus* 2'3'-cGAMP concentration using linear (y) and log (x) axes and perform a four-parameter logistic fit.

Alternative Plot - The data can also be linearized using a logit transformation. The equation for this conversion is shown below. *NOTE: Do not use %B/B_0 in this calculation.*

$$\text{logit } (B/B_0) = \ln [B/B_0/(1 - B/B_0)]$$

Plot the data as logit (B/B_0) *versus* log concentrations and perform a linear regression fit.

Determine the Sample Concentration

Calculate the B/B_0 (or %B/B_0) value for each sample. Determine the concentration of each sample by identifying the %B/B_0 on the standard curve and reading the corresponding values on the x-axis. *NOTE: Remember to account for any dilution of the original sample concentration prior to its addition to the well.* Samples with %B/B_0 values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of the standard curve. A 20% or greater disparity between the apparent concentration of two different dilutions of the same sample indicates interference, which could be eliminated by purification.

NOTE: If there is an error in the B_0 wells it is possible to calculate sample concentrations by plotting the absorbance values and back calculating sample absorbance off the standard curve.

ELISA Performance Characteristics

Representative ELISA Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples.

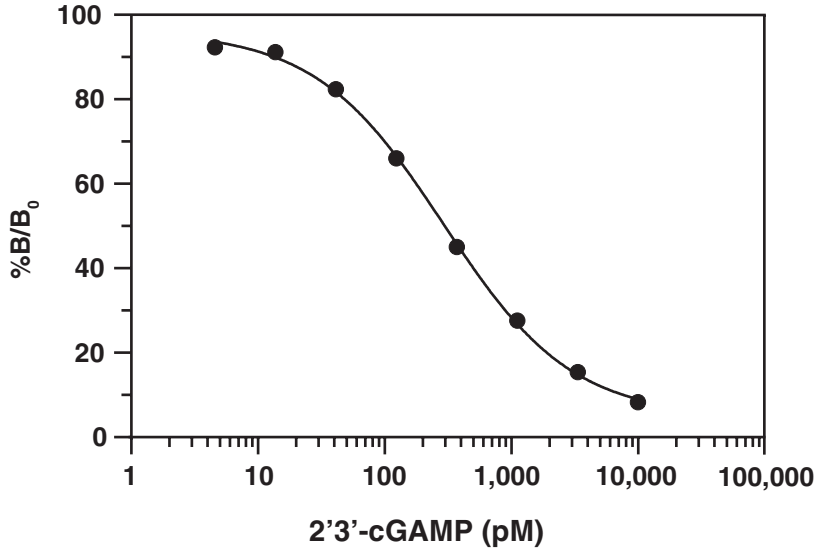
Absorbance at 450 nm (30 minutes)

Analyte Standards (pM)	Blank-subtracted Absorbance	NSB-corrected Absorbance	%B/B_0	%CV* Intra-assay Precision	%CV* Inter-assay Precision
NSB	0.000				
B_0	0.706	0.706			
10,000	0.057	0.057	8.1	6.5	15.0
3,333	0.108	0.108	15.4	6.6	3.7
1,111	0.194	0.194	27.6	6.4	3.3
370.4	0.317	0.317	45.0	2.7	3.4
123.5	0.466	0.466	65.4	6.9	2.9
41.2	0.583	0.583	82.1	8.1	6.8
13.7	0.642	0.642	90.6	31.5**	23.4**
4.6	0.651	0.651	92.0	44.2**	36.4**
TA	0.800				

Table 4. Typical results

*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve

**Evaluate data in this range with caution



Assay Range = 4.6-10,000 pM
Sensitivity (defined as 80% B/B_0) = 48.4 pM
Mid-point (defined as 50% B/B_0) = 300.1 pM
Lower Limit of Detection (LLOD) = 5.0 pM

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted in Immunoassay Buffer C (1X).

Figure 4. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing 24 replicates of two matrix controls (100% Initial Activity and Positive Control (CU-76) reactions) in a single assay.

Matrix Control (pM)	%CV
1,112.0	6.0
562.5	7.2

Table 5. Intra-assay precision

Inter-assay precision was determined by analyzing three matrix controls (cGAS reaction products) in eight separate assays on different days.

Matrix Control (pM)	%CV
904.4	4.2
395.2	9.4
181.1	9.5

Table 6. Inter-assay precision

Cross Reactivity:

Compound	Cross Reactivity
2'3'-cGAMP	100%
2'2'-cGAMP	5.2%
3'3'-cGAMP	<0.002%
Cyclic di-AMP	<0.002%
Cyclic di-GMP	<0.002%
Cyclic AMP	<0.002%
Cyclic GMP	<0.002%
AMP	<0.002%
GMP	<0.002%
ADP	<0.002%
GDP	<0.002%
ATP	<0.00002%
GTP	<0.00002%

Table 7. Cross reactivity of the cGAS Inhibitor Screening ELISA

cGAS REACTION ANALYSIS

Calculations

1. Determine the percent inhibition or percent activity for each inhibitor using one of the following equations:

$$\%\text{Inhibition} = \left[\frac{\text{(calculated 100\% initial activity concentration - calculated inhibitor activity concentration)}}{\text{calculated 100\% initial activity concentration}}\right] \times 100$$

$$\%\text{Activity} = \left[\frac{\text{(calculated inhibitor activity concentration)}}{\text{calculated 100\% initial activity concentration}}\right] \times 100$$

2. Graph the percent inhibition or percent activity as a function of inhibitor concentration to determine the IC_{50} value (the concentration at which there is 50% inhibition) of the inhibitor. Inhibition of recombinant human cGAS by cGAS Inhibitor (CU-76), is shown in Figure 7 (see page 37).

Performance Characteristics

Z' Factor:

Z' factor is a term used to describe the robustness of an assay, which is calculated using the equation below.[8]

$$Z' = 1 - \frac{3\sigma_{c+} + 3\sigma_{c-}}{|\mu_{c+} - \mu_{c-}|}$$

Where σ: Standard deviation
μ: Mean
c+: Positive control
c-: Negative control

The theoretical upper limit for the Z' factor is 1.0. A robust assay has a Z' factor >0.5. The Z' factor for Cayman's cGAS Inhibitor Screening Assay Kit was determined to be 0.73.

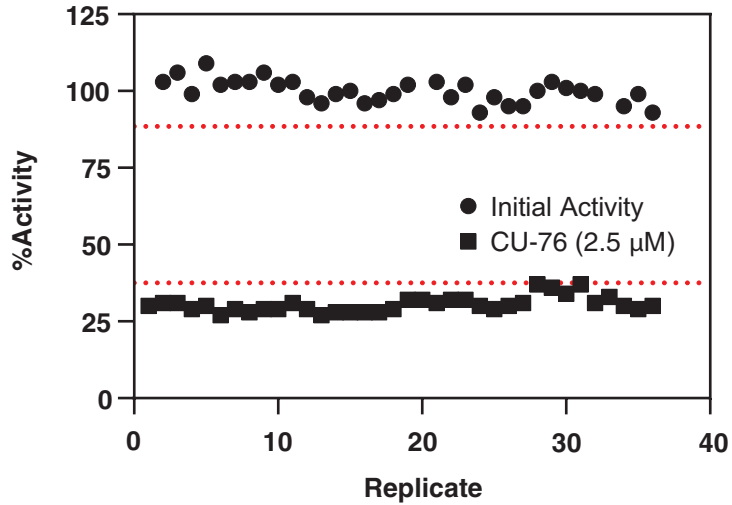


Figure 5. Typical Z' data for the cGAS Inhibitor Screening Assay Kit. Data are shown from 36 replicates each for vehicle control (Veh.) and 2.5 µM cGAS Inhibitor (CU-76) prepared as described in the kit booklet. The calculated Z' factor for this experiment was 0.73. The red dotted lines correspond to three standard deviations from the mean for each control value.

Effects of Solvents:

Compounds may be prepared in organic solvents such as DMF, DMSO, or short-chain alcohols (*e.g.*, EtOH, MeOH), as long as the final concentration of organic solvents in the assay is ≤2% for DMSO or MeOH and ≤0.1% for DMF or EtOH. A titration of organic solvents showed that the signal changes slightly with increasing solvent concentration so the proper vehicle control should be included in the assay.

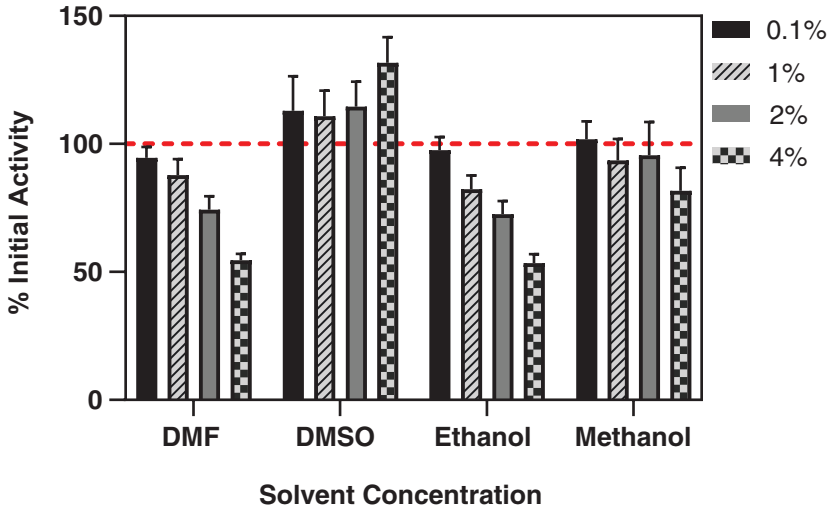


Figure 6. The effect of solvent on the readout of cGAS activity. The data are shown as the mean of multiple ELISA dilutions ± standard deviation for the indicated concentration of solvents. The red dotted line at 100% is the buffer control.

Sample Data:

The data presented here are an example of data typically produced with this kit; however, your results will not be identical to these. Do not use the data below to directly compare to your samples. Your results could differ substantially.

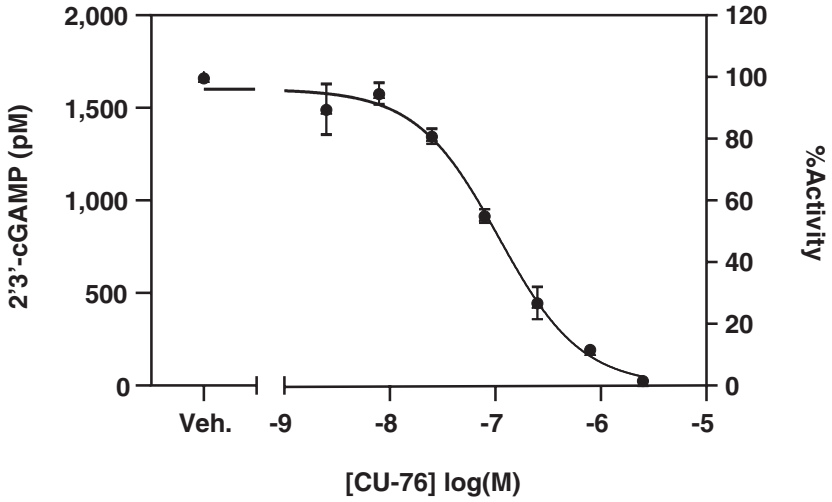


Figure 7. Inhibition of recombinant human cGAS by cGAS Inhibitor (CU-76). Data are plotted as the mean of multiple ELISA dilutions ± the standard deviation. The vehicle control (Veh.) represents 100% initial activity. The IC_{50} value of cGAS Inhibitor (CU-76) is 108 nM.

Precision:

Intra-assay precision was determined by analyzing 24-36 measurements of the background, vehicle, and 2.5 µM cGAS Inhibitor (CU-76) on the same day. The intra-assay coefficients of variation were 3.9, 4.0, and 7.7%, respectively.

Inter-assay precision was determined by analyzing inhibition curves with cGAS Inhibitor (CU-76) in separate assays on three different days at a 1:10 dilution of the reaction mixture. The inter-assay coefficient of variance for the IC_{50} value was 9.7%.

RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates/ triplicates	A. Trace organic contaminants in the water source B. ELISA plate was not pre-washed C. Poor pipetting/technique
High NSB (>10% of blank-subtracted B_0 values)	A. Poor washing B. Exposure of NSB wells to specific antiserum
Very low B_0	A. Trace organic contaminants in the water source B. Dilution error in preparing reagents
Low sensitivity	Standard is degrading
Analyses of two dilutions do not agree (*i.e.*, more than 20% difference)	A. Interfering substances are present B. ELISA plate was not pre-washed
Only TA wells develop	Trace organic contaminants in the water source
No inhibition seen with compound	A. The concentration of the compound is not high enough B. The compound is not an inhibitor of the enzyme
No 2'3'-cGAMP production detected in 100% initial activity wells	A. Enzyme or substrate was not added to the cGAS reaction B. Degradation of enzyme or substrate
No 2'3'-cGAMP production detected in unknown inhibitor wells	A. Enzyme or substrate was not added to the cGAS reaction B. Unknown inhibitor concentration is too high and inhibited all of the cGAS activity

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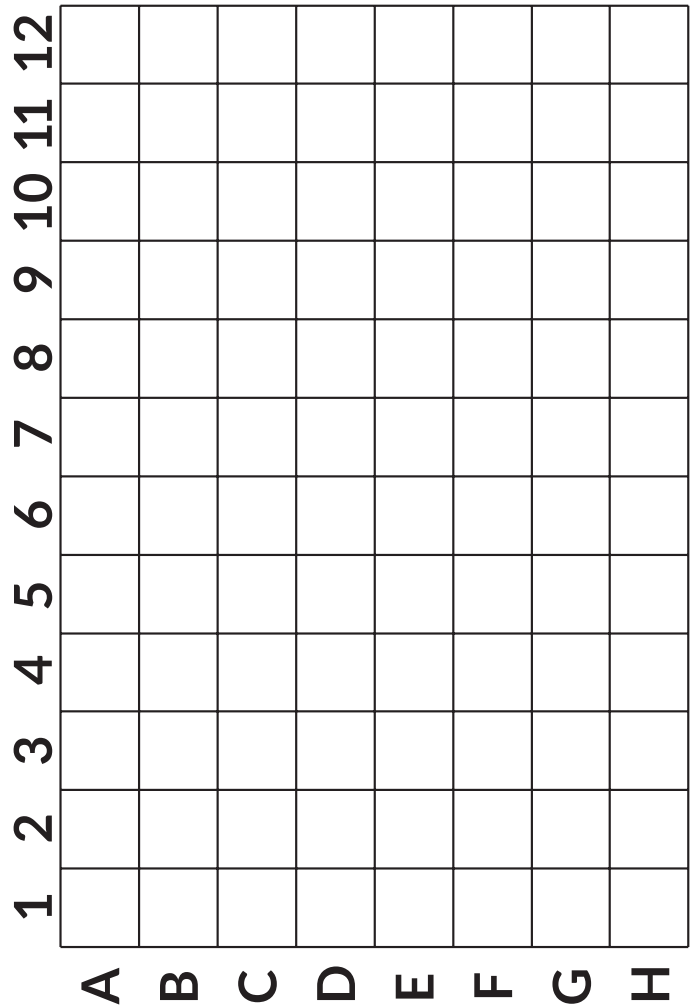

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.