cGAS TR-FRET Inhibitor Screening Assay Kit

Item No. 702120

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GENERAL INFORMATION

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
400519	cGAS TR-FRET Assay Buffer (4X)	1 vial/6 ml	-20°C
400520	cGAS TR-FRET Substrate Mix	1 vial/2.4 ml	-20°C
400521	cGAS TR-FRET Stop Solution	1 vial/2.4 ml	-20°C
400522	cGAS TR-FRET Detection Mix	1 vial/2.4 ml	-20°C
400523	cGAS Enzyme (human, recombinant)	1 vial/100 µl	-80°C
400525	cGAS TR-FRET Inhibitor (CU-76)	1 vial/20 µl	-20°C
400012	96-Well Cover Sheet	1 ea	RT
400023	Foil Plate Cover	1 ea	RT
400530 OR 600854	Half-Area 96-Well White Plate OR Low-Volume 384-Well White Plate	1 plate	RT

If any of the items listed above 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.

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.

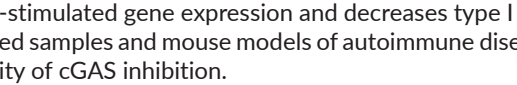
Materials Needed But Not Supplied

1. A plate reader capable of TR-FRET with an excitation wavelength of 340 nm and emission wavelengths of 615 and 665 nm
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. An orbital microplate shaker
4. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
5. Microcentrifuge tubes or dilution plates
6. Timer

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 2'3'-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.



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About This Assay

Cayman's cGAS TR-FRET Inhibitor Screening Assay Kit provides a robust and easy-to-use time-resolved Förster resonance energy transfer (TR-FRET) platform for identifying novel inhibitors of human cGAS, which is a cystosolic dsDNA sensor that detects microbial pathogens. This assay utilizes a mouse anti-rabbit monoclonal immunoglobulin G (IgG) directly labeled with a europium (Eu^{3+}) chelate as a donor molecule and fluorescently labeled 2'3'-cGAMP as the acceptor molecule. In the presence of an anti-2'3'-cGAMP antibody, the donor and acceptor are brought into close proximity resulting in a FRET from the donor to the acceptor upon excitation of the Eu^{3+}-conjugated IgG at 340 nm and an emission from the acceptor at 665 nm. In the assay, production of 2'3'-cGAMP by cGAS in the presence of dsDNA, ATP, and GTP results in the displacement of fluorescently labeled 2'3'-cGAMP from the anti-2'3'-cGAMP antibody, resulting in a loss of TR-FRET signal. Inhibition of cGAS maintains the signal. The cGAS inhibitor CU-76 is included as a positive control.

Introduction to TR-FRET

TR-FRET is based upon the principles of FRET but possesses a number of advantages that make it a superior technology for high-throughput screening. When an optically active molecule absorbs a photon, it has several options by which it may release that energy: it may release a photon of a longer wavelength (less energy) than the photon it absorbed, it may dissipate the energy as heat, or it can transfer the energy non-radiometrically to a suitable acceptor fluorophore. The latter effect is known as FRET, and it is a commonly used phenomenon in biological assays. In these assays, a donor flurophore is coupled to one binding partner and an acceptor fluorophore is coupled to the other binding partner. The binding partners are mixed in an assay well and allowed to associate. The donor fluorophore is then excited with a wavelength of light that does not excite the acceptor fluorophore, and if the molecules are within approximately 100 Å of each other, the donor fluorophore can non-radiometrically transfer the energy to the acceptor fluorophore, which will then release that photon as light at a wavelength characteristic of the acceptor fluorophore (see Figure 1 on page 9). For each assay point, the fluorescence intensity of the donor fluorophore and the acceptor fluorophore are measured, and the data are generally presented as the ratio of acceptor fluorophore intensity/donor fluorophore intensity. This methodology is particularly sensitive because the FRET efficiency decays as a function of the inverse 6^{th} power of the distance between the two fluorophores. Therefore, unassociated binding partners are unlikely to lie within the distance required for efficient FRET.

TR-FRET is an extension of FRET that utilizes a donor fluorophore with a long fluorescent half-life. These fluorophores are based upon lanthanide (most often Eu^{3+} or Tb^{3+}) chelates that have characteristically large Stokes shifts and fluorescent half-lives on the order of milliseconds. The long fluorescent lifetime allows the TR-FRET signal to be sustained for dramatically longer periods of time than standard fluorescence. This is particularly advantageous because it affords the ability to measure the TR-FRET signal after background fluorescence in the assay (*e.g.*, buffer/reagent autofluorescence) has dissipated. The increased signal:noise ratio and the diminished effects of screening compound fluorescence makes TR-FRET assays particularly useful for high-throughput screening applications.

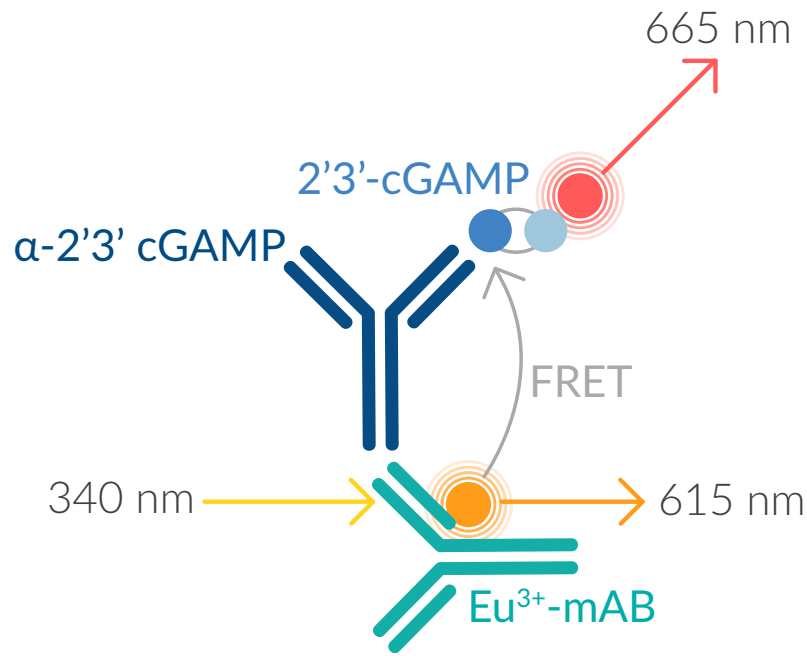


Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Sample Preparation

All inhibitors, be they small molecules, natural products, or proteins, should be prepared at a concentration that is 4X the desired final assay concentration (*e.g.*, for 4 µM final assay concentration, a 16 µM stock should be made). Test inhibitors should be prepared in cGAS TR-FRET Assay Buffer (1X) and the resulting solutions may contain up to 20% DMSO, 0.4% dimethyl formamide (DMF), or 0.4% short-chain alcohols (*e.g.*, MeOH, EtOH). The final concentration of organic solvents in the assay will then be ≤5, ≤0.1, or ≤0.1%, respectively (see **Effects of Solvents** on page 23). **See Reagent Preparation step 6, on page 11, for an example of how to prepare inhibitors.**

In addition, a solution containing the same solvent at the same concentration used for the test inhibitor and the positive control cGAS TR-FRET inhibitor, CU-76, should be prepared in cGAS TR-FRET Assay Buffer (1X). This is the vehicle control (4X).

Reagent Preparation

NOTE: Thaw all reagents on ice unless otherwise noted. However, allow reagents to warm up to room temperature immediately prior to use in the assay.

1. **cGAS TR-FRET Assay Buffer (1X)**

   Mix 2 ml of cGAS TR-FRET Assay Buffer (4X) (Item No. 400519) with 6 ml of ultrapure water to make 8 ml of cGAS TR-FRET Assay Buffer (1X). The cGAS TR-FRET Assay Buffer (1X) should be discarded if not used within the same day. Once thawed, the cGAS TR-FRET Assay Buffer (4X) may be stored at 4°C for up to one week.

2. **cGAS TR-FRET Substrate Mix**

   The vial of cGAS TR-FRET Substrate Mix (Item No. 400520) is ready to use as supplied. If all of the cGAS TR-FRET Substrate Mix will not be used at one time, aliquot the undiluted substrate mix and store at -20°C where it will be stable for at least six months.

3. **cGAS TR-FRET Stop Solution**

   The cGAS TR-FRET Stop Solution (Item No. 400521) is ready to use as supplied. If all of the cGAS TR-FRET Stop Solution will not be used at one time, aliquot the reagent and store at -20°C. Keep protected from light.

4. **cGAS TR-FRET Detection Mix**

   The cGAS TR-FRET Detection Mix (Item No. 400522) is ready to use as supplied. If all of the cGAS TR-FRET Detection Mix will not be used at one time, aliquot the reagent and store at -20°C. Keep protected from light.

5. **cGAS Enzyme (human, recombinant) Working Solution**

   cGAS Enzyme (human, recombinant) (Item No. 400523) should be thawed on ice and mixed prior to dilution. Dilute the enzyme by mixing 90 µl of cGAS Enzyme (human, recombinant) with 4,410 µl of cGAS TR-FRET Assay Buffer (1X) to make a working solution. 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 all of the cGAS Enzyme (human, recombinant) will not be used at one time, aliquot the undiluted enzyme and store at -80°C, limiting freeze-thaw cycles.

6. **cGAS TR-FRET Inhibitor (CU-76) Working Solution**

   This vial contains 20 µl of 2 mM cGAS TR-FRET Inhibitor (CU-76) (Item No. 400525) in DMSO, which can be used as a positive control. Thaw at room temperature and mix 16 µl of 2 mM cGAS TR-FRET Inhibitor (CU-76) with 44 µl ultrapure water. Then, add 20 µl of cGAS TR-FRET Assay Buffer (4X) to make a 4X inhibitor working solution (*i.e.*, 400 µM CU-76 working solution in 20% DMSO). If all of the cGAS TR-FRET Inhibitor (CU-76) will not be used at one time, aliquot the undiluted inhibitor and store at -20°C.

TR-FRET Plate Reader Settings

We recommend reading the TR-FRET assay at two wavelengths, detecting both the emission from the Eu^{3+} chelate donor at 615 nm and from the acceptor fluorophore at 665 nm. Table 1, below, provides instrument settings to be used as guidelines.

	TR-FRET-Compatible Plate Reader	
Parameter	**Flash Lamp Excitation**	**Laser Excitation**
Excitation Filter	320 nm (or 340 nm)	--
Emission Filters	615 nm (or 620 nm) and 665 nm (or 670 nm)	615 nm (or 620 nm) and 665 nm (or 670 nm)
Delay Time	90 µs	50 µs
Flash Energy Level	100% or High	100%
Number of Flashes	100	20
Window (Integration Time)	300 µs	100 µs

Table 1. Recommended TR-FRET plate reader settings

ASSAY PROTOCOL FOR 96-WELL ASSAY

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. There is no specific pattern for using the wells on the plate. However, it is necessary to have three wells designated as 100% initial activity. It is suggested that each inhibitor, including the positive control cGAS TR-FRET Inhibitor (CU-76), be assayed in triplicate. It is suggested that the contents of each well be recorded on the template sheet provided on page 29. A typical layout of samples to be measured in triplicate is shown in Figure 2, below.

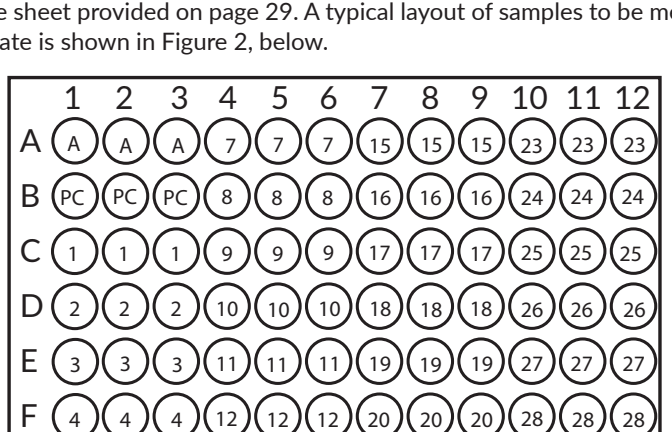


Figure 2. Sample 96-well plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- 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.

General Information

- The final volume of the assay is 120 µl in all the wells for the 96-well plate format.
- The cGAS enzyme should be kept on ice. All other reagents should be kept on ice and then warmed to room temperature immediately prior to beginning the assay unless otherwise noted.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended to assay the samples in triplicate, but it is at the user's discretion to do so.
- The assay is performed at room temperature.
- Monitor the fluorescence with an excitation wavelength of 340 nm and emission wavelengths of 615 and 665 nm.

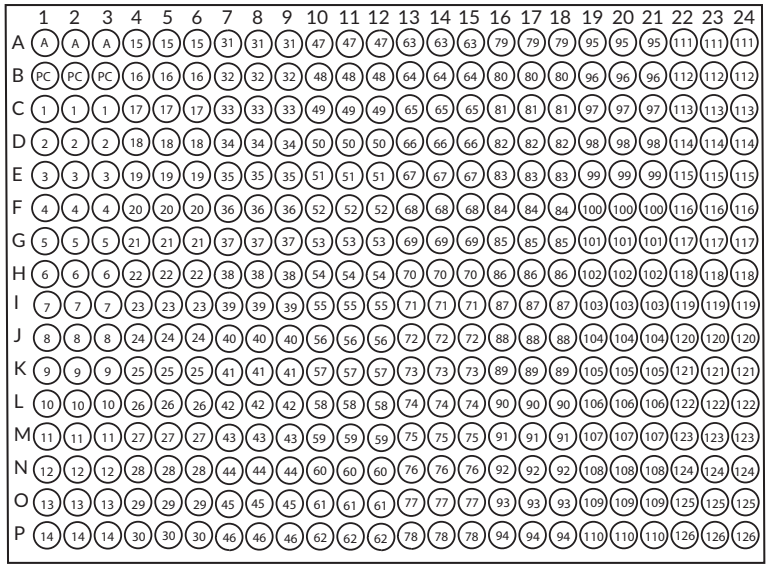
Performing the Assay: 96-Well Plate Format

1. **100% Initial Activity Wells**: add 40 µl of cGAS Enzyme working solution and 20 µl of vehicle control (4X) to three wells. If inhibitors in different solvents are to be assayed at the same time, separate sets of 100% initial activity wells should be run for each solvent.
2. **Inhibitor/Positive Control Wells**: add 40 µl of cGAS Enzyme working solution and 20 µl of test inhibitor or the 400 µM positive control, cGAS TR-FRET Inhibitor (CU-76) working solution, to three wells.
3. Incubate for 5 minutes at room temperature.
4. Initiate the reactions by adding 20 µl of cGAS TR-FRET Substrate Mix to all the 100% initial activity wells, inhibitor wells, and positive control wells being used. Mix thoroughly by pipetting up and down at least six times.
5. Cover the plate with the clear 96-Well Cover Sheet (Item No. 400012) and incubate for 30 minutes at room temperature.
6. Gently remove the plate cover and quench the reaction with 20 µl of cGAS TR-FRET Stop Solution to all wells. Mix thoroughly.
7. Add 20 µl of cGAS TR-FRET Detection Mix to all wells. Mix thoroughly.
8. Cover the plate with the Foil Plate Cover (Item No. 400023). Let equilibrate at room temperature for at least 30 minutes.
9. Gently remove the plate cover. Read on a TR-FRET-compatible plate reader. *NOTE: The same plate can be read several times without a negative effect on the assay performance, and the signal is stable for at least 24 hours at room temperature as long as the plate is sealed to prevent evaporation.*

ASSAY PROTOCOL FOR 384-WELL ASSAY

Plate Set Up

The 384-well plate(s) included with this kit is supplied ready to use. There is no specific pattern for using the wells on the plate. However, it is necessary to have three wells designated as 100% initial activity. It is suggested that each inhibitor, including the positive control cGAS TR-FRET Inhibitor (CU-76), be assayed in triplicate. A typical layout of samples to be measured in triplicate is shown in Figure 3, below.



A = 100% Initial Activity Wells
PC =Positive Control Wells
1-126 = Inhibitor Wells

Figure 3. Sample 384-well plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- 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.

General Information

- The final volume of the assay is 24 µl in all the wells for the 384-well plate format.
- The cGAS enzyme should be kept on ice. All other reagents should be kept on ice and then warmed to room temperature immediately prior to beginning the assay unless otherwise noted.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended to assay the samples in triplicate, but it is at the user's discretion to do so.
- The assay is performed at room temperature.
- Monitor the fluorescence with an excitation wavelength of 340 nm and emission wavelengths of 615 and 665 nm.

Performing the Assay: 384-Well Plate Format

1. **100% Initial Activity Wells**: add 8 µl of cGAS Enzyme working solution and 4 µl of vehicle control (4X) to three wells. If inhibitors in different solvents are to be assayed at the same time, separate sets of 100% initial activity wells should be run for each solvent.
2. **Inhibitor/Positive Control Wells**: add 8 µl of cGAS Enzyme working solution and 4 µl of test inhibitor or the 400 µM positive control, cGAS TR-FRET Inhibitor (CU-76) working solution, to three wells.
3. Incubate for 5 minutes at room temperature.
4. Initiate the reactions by adding 4 µl of cGAS TR-FRET Substrate Mix to all the 100% initial activity wells, inhibitor wells, and positive control wells being used. Mix thoroughly by pipetting up and down at least six times.
5. Cover the plate with the clear 96-Well Cover Sheet (Item No. 400012) and incubate for 30 minutes at room temperature.
6. Gently remove the plate cover and quench the reaction with 4 µl of cGAS TR-FRET Stop Solution to all wells. Mix thoroughly.
7. Add 4 µl of cGAS TR-FRET Detection Mix to all wells. Mix thoroughly.
8. Cover the plate with the Foil Plate Cover (Item No. 400023). Let equilibriate at room temperature for at least 30 minutes.
9. Gently remove the plate cover. Read on a TR-FRET-compatible plate reader. *NOTE: The same plate can be read several times without a negative effect on the assay performance, and the signal is stable for at least 24 hours at room temperature as long as the plate is sealed to prevent evaporation.*

ANALYSIS

Calculations

TR-FRET data are typically calculated and presented ratiometrically using the fluorescence intensities (FI) at 665 nm and 615 nm according to the following formula:

$$[(FI_{665\ nm}/FI_{615\ nm}) \times 1{,}000]$$

OPTIONAL: To accurately determine the IC_{50} value of an inhibitor, it is highly recommended to run a standard curve using 2'3'-cGAMP (Item No. 19887; not included in this kit) at concentrations ranging from 0.3 nM to 100 µM prepared in cGAS TR-FRET Buffer (1X). The data can be fit to a four-parameter logistic equation as shown in Figure 4 (see page 20). The standard curve can be used to interpolate the amount of 2'3'-cGAMP produced by cGAS in the reaction, and can be graphed as a function of inhibitor concentration in log units. Inhibition of recombinant human cGAS by the positive control cGAS TR-FRET Inhibitor (CU-76) is shown in Figure 5 (see page 21).

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 TR-FRET Inhibitor Screening Assay Kit was determined to be 0.80.

Sample Data:

The data presented here is 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.

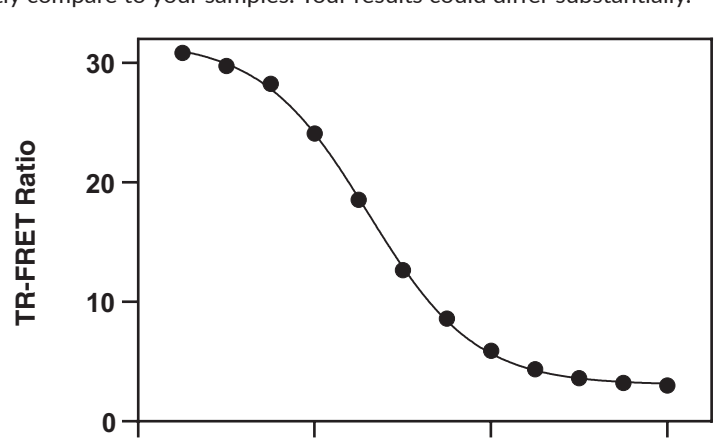


Figure 4. Typical standard curve (optional). TR-FRET ratios are plotted as the mean of triplicate measurements ± the standard deviation *versus* the concentration of the 2'3'-cGAMP standard ranging from 0.3 nM to 100 µM. 2'3'-cGAMP is sold separately.

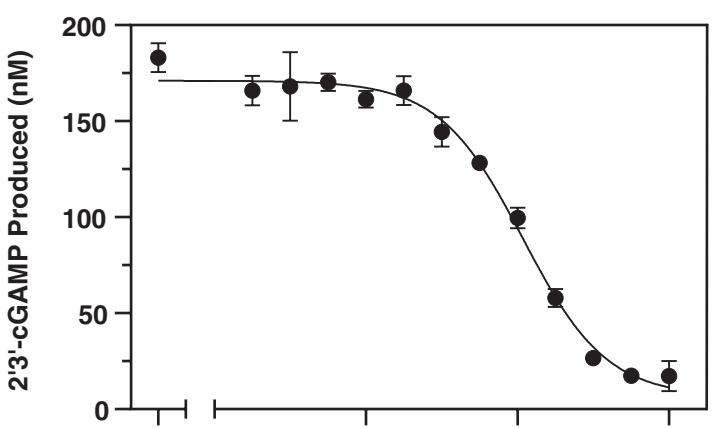


Figure 5. Inhibition of recombinant human cGAS by cGAS TR-FRET Inhibitor (CU-76). Data are plotted as the mean of triplicate measurements ± the standard deviation. The vehicle control (Veh.) represents 100% initial activity. The IC_{50} value of cGAS TR-FRET Inhibitor (CU-76) is 1,225 nM.

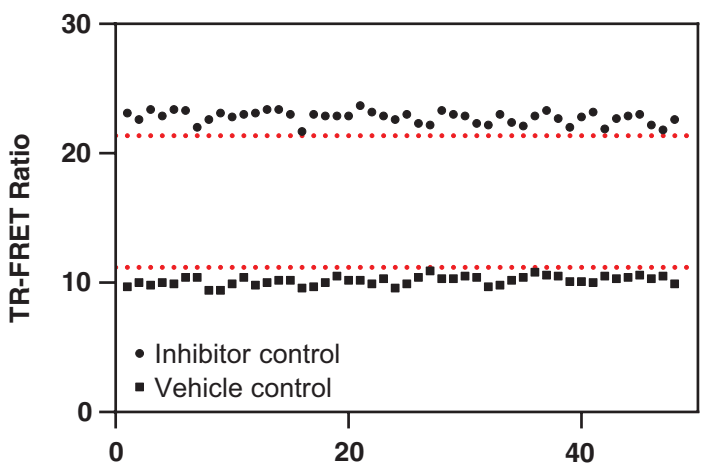


Figure 6. Typical performance data for the cGAS TR-FRET Inhibitor Screening Assay Kit. Data are shown from 48 replicates each for vehicle control and 100 µM cGAS TR-FRET Inhibitor (CU-76) prepared as described in the kit booklet. The calculated Z′ factor for this experiment was 0.80. The red lines correspond to three standard deviations from the mean for each control value.

Effects of Solvents:

Compounds may be prepared in organic solvents such as DMSO, DMF, or short-chain alcohols (*e.g.*, MeOH, EtOH), as long as the final concentration of organic solvents in the assay is ≤5, ≤0.1, or ≤0.1%, respectively. A titration of organic solvents showed that signal decreases with increasing solvent concentration so the proper vehicle control should be included in the assay.

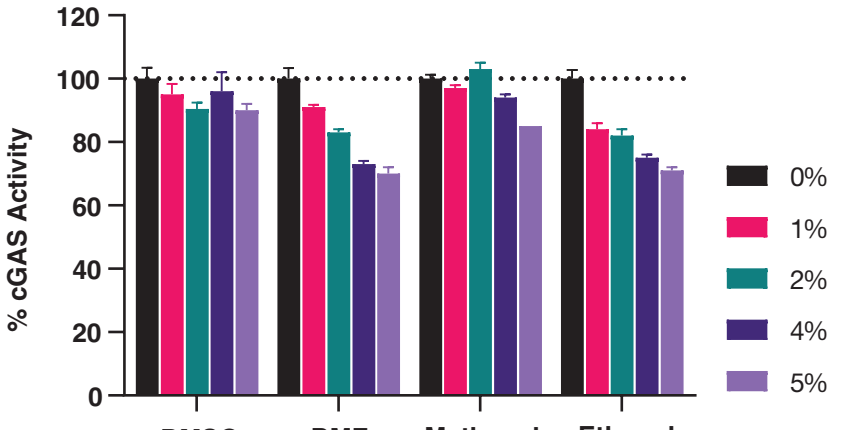


Figure 7. The effect of solvent on the readout of cGAS activity. The data are shown as the mean ± standard deviation for triplicate reactions containing the indicated concentration of solvents.

Precision:

Intra-assay precision was determined by analyzing 24 measurements of the vehicle control and the cGAS TR-FRET Inhibitor (CU-76) on the same day. The intra-assay coefficient of variation was 4% for both. The intra-assay coefficient of variation for the IC_{50} value of six inhibition curves performed on the same day was 19%.

Inter-assay precision was determined by analyzing inhibition with the cGAS TR-FRET Inhibitor (CU-76) in five separate assays on different days. The inter-assay coefficient of variation for the IC_{50} value was 15%.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Bubble in the well B. Poor pipetting/technique	A. Carefully tap the side of the plate with your finger to remove bubbles B. Be careful not to splash the contents of the wells
Low fluorescence signal	A. Incompatible sample matrix B. cGAS enzyme handled improperly C. Monochromater-based instrument used for data acquisition	A. Test sample matrix for interference before running samples in the assay B. Keep the enzyme frozen at -80°C until ready to use; thaw enzyme and keep on ice until just prior to use in the assay C. Analyze the assay using a filter-based plate reader

References

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2. Wu, J., Sun, L., Chen, X., *et al.* Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. *Science* **339(6121)**, 826-830 (2013).
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4. Guey, B., Wischnewski, M., Decout, A., *et al.* BAF restricts cGAS on nuclear DNA to prevent innate immune activation. *Science* **369(6505)**, 823-828 (2020).
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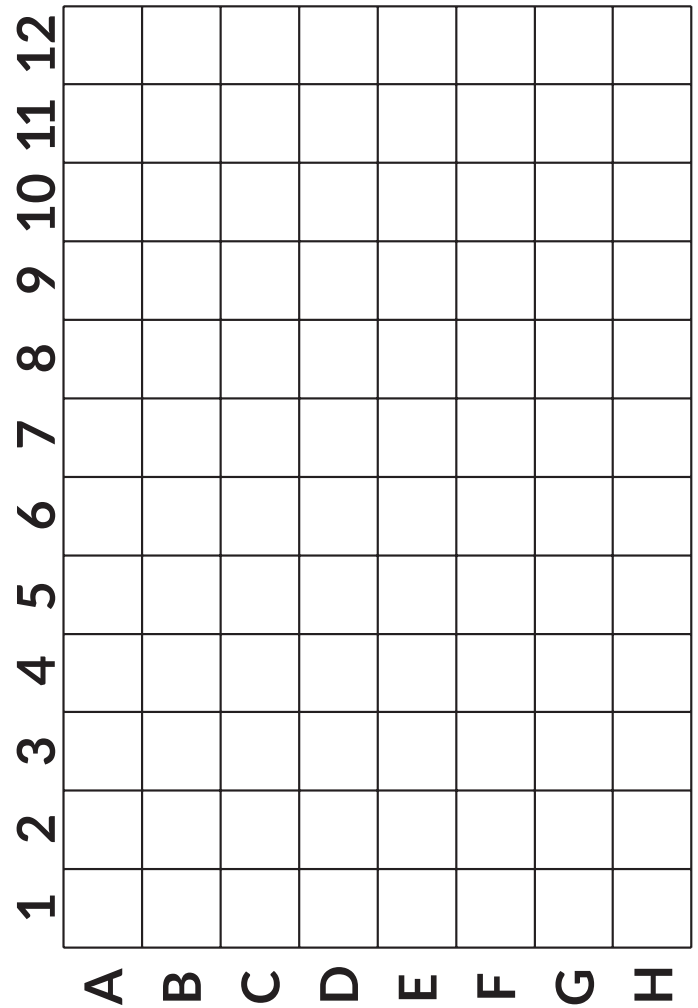
7. Yum, S., Li, M., and Chen, Z.J. Old dogs, new trick: classic cancer therapies activate cGAS. *Cell Res.* **30(8)**, 639-648 (2020).
8. Zhang, J.-H., Chung, T.D.Y., and Oldenburg, K.R. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J. Biomol. Screen.* **4(2)**, 67-73 (1999).

	Initial Activity Wells	Positive Control Wells	Inhibitor Wells	Standard Wells (Optional)	Comments
cGAS Enzyme Working Solution	40 µl	40 µl	40 µl	--	--
Vehicle Control (4X)	20 µl	--	--	--	--
400 µM Positive Control	--	20 µl	--	--	--
Test Inhibitor (4X)	--	--	20 µl	--	--
0.3 nM to 100 µM 2'3'-cGAMP Standards	--	--	--	80 µl	Prepare in cGAS TR-FRET Assay Buffer (1X)
Incubate at room temperature for 5 minutes					
Substrate Mix	20 µl	20 µl	20 µl	--	Mix thoroughly
Cover and incubate at room temperature for 30 minutes					
Stop Solution	20 µl	20 µl	20 µl	20 µl	Mix thoroughly
Detection Mix	20 µl	20 µl	20 µl	20 µl	Mix thoroughly
Cover with foil plate cover and incubate at room temperature for 30 minutes					
Remove foil plate cover and measure TR-FRET (ex 340 nm/em 615 and 665 nm)					

Table 2. Assay summary for the 96-well plate format

	Initial Activity Wells	Positive Control Wells	Inhibitor Wells	Standard Wells (Optional)	Comments
cGAS Enzyme Working Solution	8 µl	8 µl	8 µl	--	--
Vehicle Control (4X)	4 µl	--	--	--	--
400 µM Positive Control	--	4 µl	--	--	--
Test Inhibitor (4X)	--	--	4 µl	--	--
0.3 nM to 100 µM 2'3'-cGAMP Standards	--	--	--	16 µl	Prepare in cGAS TR-FRET Assay Buffer (1X)
Incubate at room temperature for 5 minutes					
Substrate Mix	4 µl	4 µl	4 µl	--	Mix thoroughly
Cover and incubate at room temperature for 30 minutes					
Stop Solution	4 µl	4 µl	4 µl	4 µl	Mix thoroughly
Detection Mix	4 µl	4 µl	4 µl	4 µl	Mix thoroughly
Cover with foil plate cover and incubate at room temperature for 30 minutes					
Remove foil plate cover and measure TR-FRET (ex 340 nm/em 615 and 665 nm)					

Table 3. Assay summary for the 384-well plate format



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.