2',3'-Cyclic GAMP Competitive ELISA Kit

Catalog Number EIAGAMP (96 tests)

Pub. No. MAN0018782 **Rev** B.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The 2',3'-Cyclic GAMP Competitive ELISA Kit is designed to detect and quantify the level of 2',3'-cGAMP in lysed cells and tissue, and tissue culture media samples. It has been matrix tested for EDTA plasma.

Contents and storage

Kit and components are shipped at -20°C. Upon receipt, store the kit at -20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity
Antibody Coated Wells, 96-well strip-well plate coated with goat anti-rabbit IgG	1 plate
2',3'-Cyclic GAMP Standard; 1,000 pmol/mL	125 µL
2',3'-Cyclic GAMP Antibody	3 mL
2',3'-Cyclic GAMP Conjugate	3 mL
Assay Buffer Concentrate (5X)	28 mL
Wash Buffer Concentrate (20X)	30 mL
TMB (Tetramethylbenzidine) Substrate	11 mL
Stop Solution; contains 1 M HCl, **CAUSTIC**	5 mL
Plate Sealer	1

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm (preferably with correction between 570 nm and 590 nm).
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 15 mL of Wash Buffer Concentrate (20X) with 285 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and the 1X Wash Buffer in the refrigerator. Use the diluted buffer within 3 months.

Prepare 1X Assay Buffer

1. Dilute 14 mL of Assay Buffer Concentrate (5X) with 56 mL of deionized or distilled water. Label as 1X Assay Buffer.

Store the concentrate and the 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 4°C for 3 months.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Prepare samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application. These procedures may require optimization based on the sample type. A study of the literature is recommended.

Use all samples within 2 hours of dilution, or store at -20°C or lower until ready to perform assay.

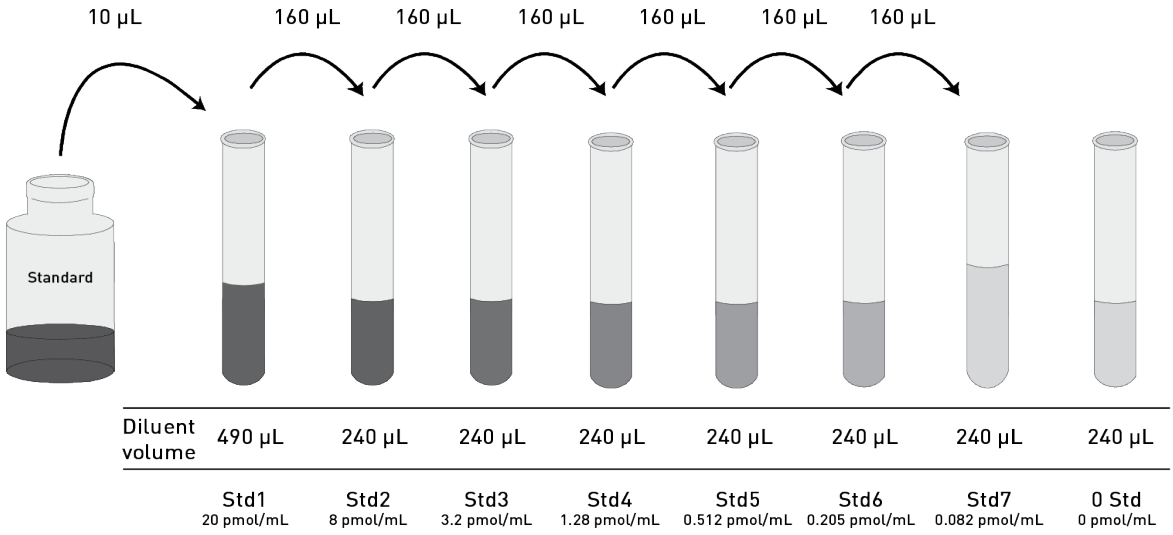
Sample type	Procedure
Tissue	• Freeze in liquid nitrogen and store at ≤-70°C if analysis is not to be carried out immediately. • Grind frozen tissue in mortar under liquid nitrogen until it is a fine powder. Weigh powdered tissue. • Lyse with suitable lysis buffer on ice and then centrifuge at ≥600 x g at 4°C for 15 minutes. • Collect the supernatant and run in the assay after diluting into 1X Assay Buffer or store frozen at ≤-70°C.
Cells	**Note:** Ensure lysis buffer contains EDTA to minimize transition metal activated cyclic nucleotide hydrolysis. Some cell types are extremely hardy and the end user should optimize lysis conditions, utilizing methods such as freeze-thaw cycles, ultrasonic treatments, or alternate lysis buffers to fully lyse cells. • Isolate cells from media prior to lysing. • Lyse with suitable lysis buffer on ice and then centrifuge at ≥ 600 x g at 4°C for 15 minutes. • Collect the supernatant and run in the assay after diluting into 1X Assay Buffer or store frozen at ≤-70°C.
Plasma, EDTA	Dilute plasma samples ≥ 1:5 in 1X Assay Buffer.
Tissue Culture Media	TCM samples should be read off standard curve generated in TCM.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Important: The 2′,3′-Cyclic GAMP Standard contains an organic solvent. Pipette the Standard up and down several times to wet the pipet tip before transfer to insure that volumes are dispensed accurately.

1. Add 10 µL of 2′,3′-Cyclic GAMP Standard to 1 tube containing 490 µL of 1X Assay Buffer and label as 20 pmol/mL 2′,3′-Cyclic GAMP.
2. Add 160 µL 1X Assay Buffer to each of 7 tubes labeled as follows: 8, 3.2, 1.28, 0.512, 0.205, 0.082, and 0 pmol/mL 2′,3′-Cyclic GAMP.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**. Discard any remaining reconstituted standard. Store 1X Assay Buffer at 4°C.

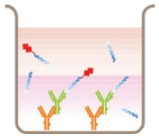


Perform ELISA (Total assay time: 2.5 hours)

IMPORTANT! Perform a standard curve with each assay.

Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store desiccated at 2°C to 8°C for future use. The silica pack in the bag keeps the plate dry, and turns from blue to pink if the bag is not properly sealed.

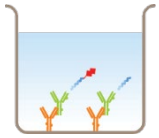


Bind antigen

a. Add 50 µL of samples or standards (see "Prepare samples" on page 2) to the appropriate wells.
b. Add 75 µL of 1X Assay Buffer to the wells for detecting non-specific binding (NSB).
c. Add 50 µL of 1X Assay Buffer into the wells for detecting maximum binding (B0 or zero standard).
d. Add 25 µL 2',3'-Cyclic GAMP Conjugate to each well.
e. Add 25 µL 2',3'-Cyclic GAMP Antibody to each well, except NSB wells.
f. Tap the side of the plate to mix. Cover the plate with a plate sealer and incubate for 2 hours at room temperature with shaking.

Note: If the plate is not shaken the signal bound will be approximately 35% lower.

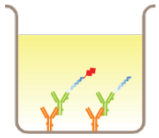
g. Thoroughly aspirate the solution and wash wells 4 times with 300 µL of 1X Wash Buffer.



Add chromogen

a. Add 100 µL TMB Substrate to each well. The substrate solution will begin to turn blue.
b. Incubate for 30 minutes at room temperature.

Note: TMB should not touch aluminum foil or other metals.



Add stop solution

Add 50 µL Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 10 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Calculate the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals lower than that of the highest standard in 1X Assay Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (examples)

The following data were obtained for the standards over the range of 0–20 pmol/mL of 2',3'-Cyclic GAMP Standard.

2',3'-Cyclic GAMP Standard (pmol/mL)	Net OD (450 nm)	%B/B0
NSB	0.000	-
20	0.199	12.8
8	0.352	22.7
3.2	0.557	35.9
1.28	0.811	52.2
0.512	1.074	69.1
0.205	1.309	84.3
0.082	1.417	91.2
B0	1.553	100

Note: The NSB gave a Mean OD value of 0.091.

Intra-assay precision

Three samples were diluted and run in replicates of 20 to determine precision of the calculated cGAMP concentrations within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pmol/mL)	1.08	5.14	10.4
%CV	6.2	6.1	5.7

CV = Coefficient of Variation

Inter-assay precision

Three samples were diluted and run in duplicates in 20 assays run by multiple operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pmol/mL)	1.00	4.92	9.97
%CV	8.5	6.7	8.0

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

Human EDTA plasma samples from healthy individuals were spiked with standard and diluted in 1x Assay Buffer. Concentrations were compared to a similarly spiked control of 1x Assay buffer. Recovery for plasma diluted 1:5-1:40 averaged 93.7%.

Specificity

The following samples were tested in the assay and cross reactivity was calculated at the 50% binding point.

Sample	Cross reactivity (%)
2',3'-cGAMP	100
2'2'-cGAMP (synthetic)	1.97
3'3'-cGAMP (bacterial)	0.03
2'3'-c-di-AMP2 (synthetic)	<0.01
cAMP	<0.01
AMP	<0.01
cGMP	<0.01

Interferents

The following detergents were tested in the assay as possible interfering substances and interference was calculated as a measured change in concentration compared to a 1X Assay Buffer- spiked standard.

Addition	Change in Measured 2',3'-cGAMP (%)
Chaps, 0.5%	-4.1
CTAC, 1.0%	-0.1
NP-40, 1.0%	+3.9
SDS, 0.02%	+8.8
Tween 20, 0.25%	-2.2
TritonX-100, 2.0%	+0.7

Linearity of dilution

Linearity was determined taking two cell lysate samples, one spiked with a low cGAMP level of 1.35 pmol/mL and one spiked with a higher level of 10.32 pmol/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High sample	Low sample	Expected Conc. (pmol/mL)	Observed Conc. (pmol/mL)	% Recovery
80%	20%	8.53	8.72	102.2%
60%	40%	6.73	6.99	103.9%
40%	60%	4.94	4.55	92.2%
20%	80%	3.14	3.41	108.4%
			Mean recovery	**101.7%**

Sensitivity

The minimum detectable dose of 2',3'-Cyclic GAMP is 0.04 pmol/mL This was determined by adding two standard deviations to the mean OD obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

REF Catalog Number | LOT Batch code | Temperature limitation | Use by | Manufacturer | Consult instructions for use | Caution, consult accompanying documents

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The information in this guide is subject to change without notice.