Development and Validation of a 2’3’-cGAMP ELISA

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Developed in collaboration with BioLog - LIFE SCIENCE INSTITUTE -

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Abstract

Cyclic GMP-AMP synthase (cGAS) is a cytosolic DNA sensing enzyme that detects the presence of nucleic acids in the cytosol of mammalian cells as an indicator of bacterial or viral infection.[1] cGAS catalyzes the synthesis of a second messenger, 2’3’-cyclic GMP-AMP (2’3’-cGAMP), from cytosolic ATP and GTP in response to dsDNA binding. 2’3’-cGAMP then binds tightly to the adaptor protein stimulator of interferon genes (STING), resulting in the recruitment of TANK-binding kinase 1 (TBK1) and subsequent interferon regulatory factor 3 (IRF3) phosphorylation.[2] IRF3 induces the transcription and translation type I interferon, a potent antiviral cytokine.[3] Activation of cGAS and the production of 2’3’-cGAMP are important in host defense, but also may play role in autoimmune or inflammatory diseases. Modulation of cGAS activity, with subsequent inhibition of induction of 2’3’-cGAMP formation is an active target of pharmacological intervention.[4]

This report will focus on the development and validation of a novel 2’3’-cGAMP enzyme linked immunoassay (ELISA). The data provided will demonstrate its value as an enzymatic readout from cell lysates and purified human recombinant cGAS.

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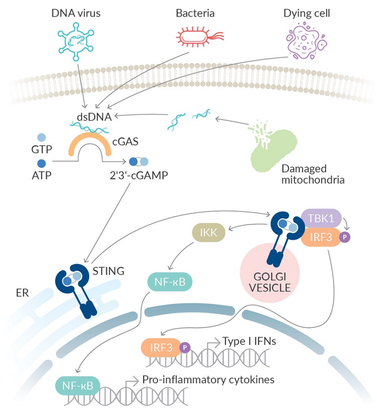
cGAS Pathway

The cGAS-STING pathway is activated by an exogenous (DNA/RNA virus or bacteria/dead cells/tumor cell) or an endogenous (mtDNA released by stressed mitochondria) agonistic DNA. cGAS, consisting of a two-lobed catalytic domain and an extended N-terminal domain, synthesizes the noncanonically linked dinucleotide 2’3’-cGAMP in which DNA stands sandwiched between the two cGAS promoters recognizing their own DNA thread during its active and stable confirmation. The second messenger 2’3’-cGAMP binds to stimulator of interferon genes (STING) dimers residing at the endoplasmic reticulum (ER) and translocates to ER-Golgi intermediate compartments, leading to STING activation.

Upon activation, TANK-binding kinase-1 (TBK1) phosphorylates the CTT of STING and recruits interferon regulatory factors (IRFs) for phosphorylation, IRF dimerization, and nuclear translocation. This leads to the activation of downstream signaling and the transcription of target genes, including type I IFNs and ISGs (IFN-stimulated genes), in addition to NF-κB transcription factors.



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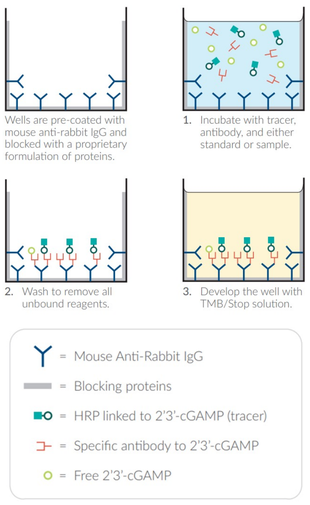
ELISA schematic



SPECIFICTY CHARACTERISTICS

Compound	Cross Reactivity
2’3’-cGAMP	100%
2’2’-cGAMP	0.8%
3’3-cGAMP	<0.01%
cyclic di-AMP	<0.01%
cyclic di-GMP	<0.01%
cGMP	<0.01%
cAMP	<0.01%
ATP	<0.01%
GTP	<0.01%

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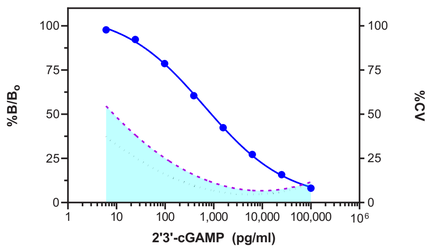
2’3’-cGAMP ELISA Validation & Performance Characteristics



%CV Intravalidation
%CV Intervalidation

Assay Range = 6.1 - 100,000 pg/ml (148.3 nM-9 pM)
Sensitivity (defined as 80% B/B_0) = 85.3 pg/ml (126 pM)
Mid-point (defined as 50% B/B_0) = 907.7 pg/ml (1.35 nM)
Lower Limit of Detection = 9.6 pg/ml (14.2 pM)

Intra-assay precision was determined by analyzing 24 replicates of three matrix controls (spiked M-PER™ samples) in a single assay.

Matrix Control (pg/ml)	%CV
7,165	8.4
828	14.5
102	21.3

Inter-assay precision was determined by analyzing replicates of three matrix controls (spiked M-PER™ samples) in separate assays over several days.

Matrix Control (pg/ml)	%CV
7,530	9.6
811	9.6
72.2	18.7

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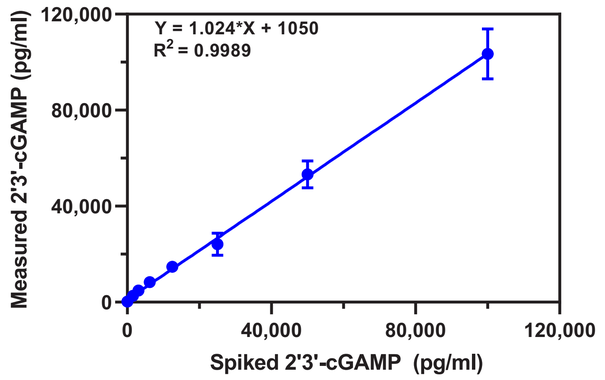
Spike & Recovery of 2’3’-cGAMP in THP-1 Cell Lysates



THP-1 cell lysate in M-PER™ (Thermo Scientific) was spiked with different amounts of 2’3’-cGAMP, serially diluted with Immunoassay Buffer C, and analyzed using the 2’3’-cGAMP ELISA. The error bars represent standard deviations from multiple dilutions of each sample.

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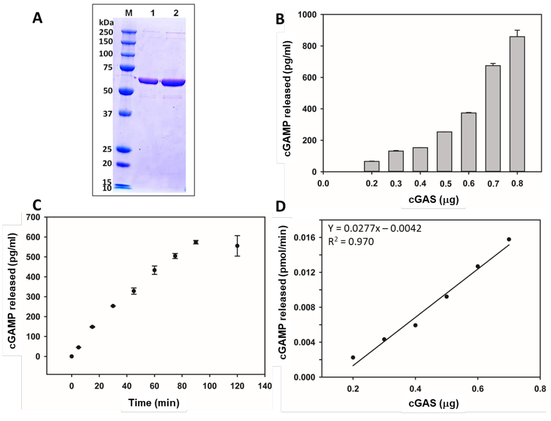
cGAS Characterization



Analysis of active human cGAS enzyme (Item No 22810). (**A**) Recombinant N-terminal His-tagged cGAS (full length, 2-522) expressed in *E. coli*. Lane 1- Marker, 2 and 3 – 2 and 4 µg of purified cGAS, respectively. Measurement of cGAMP released by (**B**) increasing concentration of cGAS enzyme assayed for 30 min and (**C**) increasing time period of cGAMP using 0.5 µM cGAS (**D**) The specific activity of cGAS was measured by plotting the rate of released cGAMP vs individual concentration of cGAS using 2’,3’-cGAMP ELISA Kit (Item 501700). One unit of cGAS produces 1 pmole of cGAMP per minute at 37°C in 80 mM Tris-HCl, pH 7.5, containing 200 mM NaCl, 20 µM $ZnCl_2$, and 20 mM $MgCl_2$ with 4 µg circular DNA (3623 bp) and 0.25 mM each of GTP and ATP

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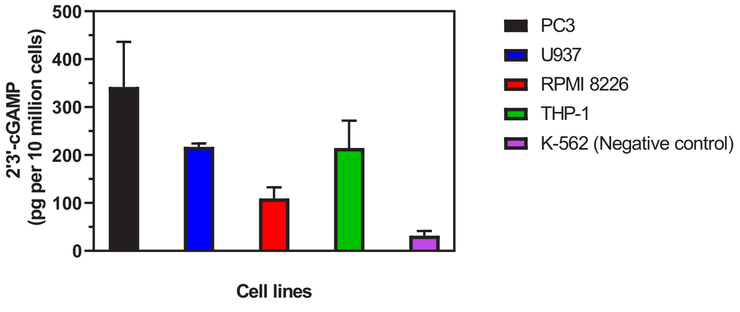
Quantification of 2’3’-cGAMP in Cell Lysates by ELISA



PC3 cells were cultured in F-12K media; RPMI 8226, U937, and THP-1 cells were cultured in RPMI 1640 media; K562 cells were cultured in IMDM media. All media contained 10% fetal bovine serum and 1% polysaccharide. Cells were lysed with M-PER™ (Thermo Scientific) at a concentration of 25 million cells per 2 ml lysis buffer. Clarified cell lysates were quantified using the 2’3’-cGAMP ELISA (Item No. 501700).

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Conclusions

The 2’3’-cGAMP ELISA is a sensitive and selective assay for measuring a wide range of 2’3’-cGAMP concentrations in cell-based and *in vitro* experiments, allowing researchers to identify cGAS modulating compounds. This assay is a valuable tool to monitor 2’3’-cGAMP released from the cGAS-STING pathway.

Acknowledgements

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References

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