STING HTRF offer to bridge innate and adaptive immunity

cGAS-STING SIGNALING PATHWAY FROM A TO Z

By controlling the production of Interferon beta, the cGas-STING pathway acts as a bridge between innate and adaptive immunity, thereby facilitating anti-tumor immunity. Thus, the cGAS-STING pathway has emerged as a potential therapeutic target in cancer. Indeed, recent promising outcomes in eliciting anti-tumor immunity paved the way for exploring the anti-cancer potential of non-canonical cyclic di-nucleotides analogs (Ramanjulu et al, Nature 2018).

This brochure outlines the use of HTRF to investigate the cGas-Sting pathway, in the context of innate immunity, and the TCR pathway, in the context of adaptive immunity.

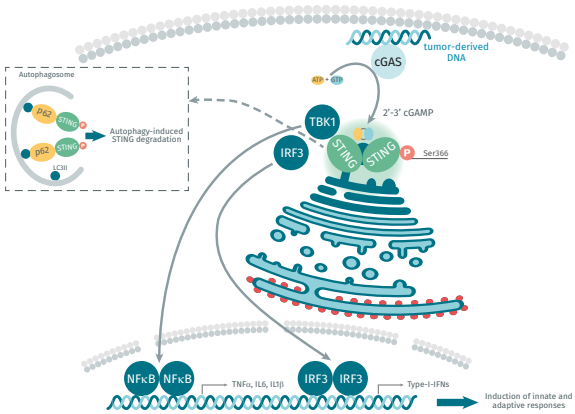
cisbio
INTERACTION IS EVERYTHING

Novel HTRF platform to delineate molecular mechanisms of STING pathway modulators

Nathalie Grégor, Cédric Chouvet, Stéphanie Douzon, Julie Vallaghé, Stéphane Martinez, Eric Trinquet, Fabienne Charrier-Savournin
Cisbio Bioassays Codolet, France

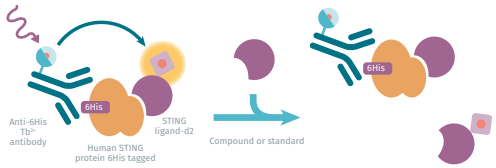
INTRODUCTION Immunotherapies targeting adaptive immune response have demonstrated unprecedented clinical efficacy in cancer treatment, especially with immune checkpoint inhibitors blocking CTLA4 or PD1. Now new drugs, which act on the innate immune system to boost the effects of the adaptive system, are being developed. In this area, the cGAS–STING pathway has been proposed to enhance anti tumor immunogenicity. We know that Tumor-derived DNA activates the cyclic GMP-AMP synthase (cGAS). This leads to the production of 2'3' cGAMP, a cyclic dinucleotide (CDN), which then binds to STING proteins. Phosphorylated STING next interacts with TBK1, leading to the recruitment and activation of the IRF3 dimer. Nuclear translocation of IRF3 leads to the transcription of genes encoding IFN-α/β. In addition, the STING pathway controls NF-κB dependent inflammatory cytokine expression. As a negative feedback loop, the DNA-stimulated cGAS-STING-TBK1 pathway also triggers STING protein degradation through a p62 SQSTM1 associated autophagy process.

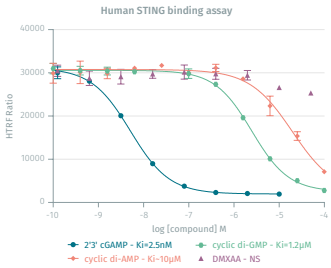
Here we provide a detailed pharmacological evaluation of the well-known STING binders 2'3' cGAMP, c-diGMP, and c-diAMP, using the new HTRF human STING biochemical assay. We also demonstrate that the new HTRF phospho and total STING assays, along with the phospho-TBK1 and the phospho-IRF3 assays, enable an in-depth understanding of STING signaling. Finally, we show a dose-dependent IFNβ secretion upon cGAMP-induced STING activation using the HTRF IFNβ assay.



BIOCHEMICAL HUMAN STING BINDING ASSAY

An HTRF competition format which uses a STING ligand-d2, a 6His tagged human STING WT protein, and an anti-6His Cryptate-labeled antibody. STING binders compete with the STING ligand-d2, thus preventing FRET from occurring.



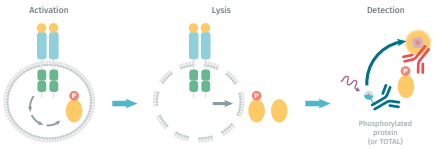


STING binders	Ki
2-3 cGAMP	2.5 nM
c-diGMP	1.2 µM
c-diAMP	>10 µM

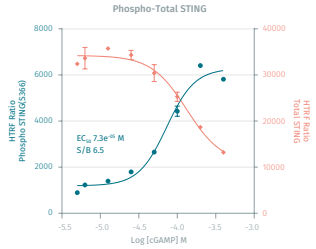
The Ki calculated for various CDN are in agreement with previously reported values (Zhang *et al* 2013). As expected, the DMXAA compound (purple triangles), which is specific for the mouse STING, does not compete with the binding of STING ligand- d2 to the human STING protein.

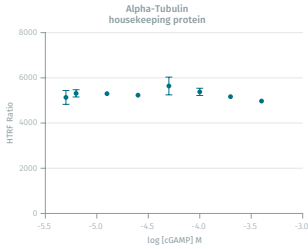
CELL-BASED HUMAN PHOSPHO & TOTAL STING

HTRF phospho and total assays use 2 labeled antibodies either with donor or acceptor fluorophores. Both HTRF assays are sandwich immunoassay which means that the intensity of the FRET signal (HTRF Ratio) is directly proportional to the concentration of the protein in the lysate.



Human THP1-R232 cells (Invivogen) were seeded at 400,000 cells/well, then stimulated with increasing concentrations of 2'3' cGAMP for 4 hours. Following a two-plate protocol for suspension cells, phospho-STING or total STING were detected by HTRF, as well as the Alpha-tubulin housekeeping protein.



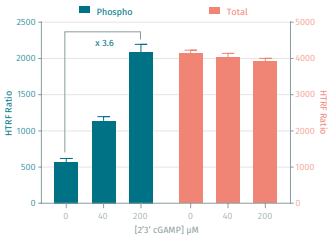


The activation of the STING pathway is revealed by a strong STING phosphorylation, associated with a down-regulation of its expression level in agreement with autophagy mediated degradation, whereas Alpha-tubulin remains stable under the same conditions. Moreover, the EC_{50} of cGAMP in cell-based phospho-STING experiment averages 75 µM, compared to 5nM established using the biochemical HTRF STING binding assay. This result indicates that 2'3' cGAMP is less potent in THP1 cells, suggesting a lack of compound permeability or a rapid degradation mechanism by the phosphodiesterase ENPP1.

DOWNSTREAM CELL-BASED ASSAYS: PHOSPHO & TOTAL TBK1 AND IRF3

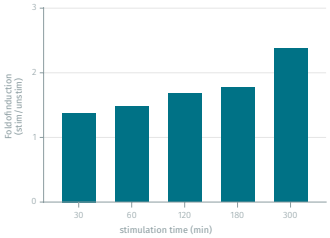
Mouse liver resident macrophage ImKC cells were seeded at 200,000 cells/well, and stimulated with 2'3' cGAMP for 1 hour.

2'3' cGAMP induces TBK1 phosphorylation in ImKC cells



Human epithelial MCF7 were seeded at 125,000 cells/well, and stimulated with 2'3' cGAMP (75 µM).

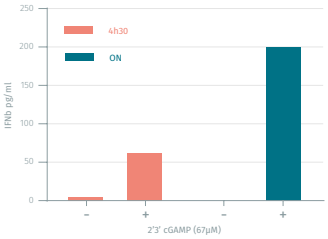
2'3' cGAMP induces IRF3 phosphorylation in MCF7 cells



QUANTIFICATION OF HUMAN IFNβ SECRETION

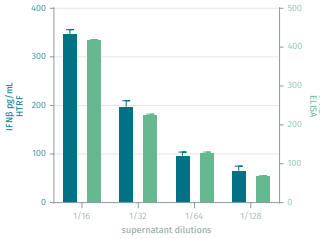
Human PBMC were seeded at 400,000 cells/well, and stimulated with 2'3' cGAMP for 4h30 and ON.

2'3' cGAMP induces IFNβ secretion as early as 4h30 post-incubation



Human THP1 cells were seeded at 400,000 cells/150 µL, and stimulated with LPS 20 µg/mL overnight.

Quantification of IFNβ by HTRF is well correlated with ELISA



CONCLUSION

To meet the needs of new STING- targeting strategies, Cisbio provides a versatile panel of biochemical and cell-based HTRF assays enabling an in-depth investigation of compounds modulating the STING pathway.

Kits Cisbio	References
HTRF human STING binding kit	64PDSTGPEG
HTRF phospho-STING (S366)	64STGPEG
HTRF total-STING	64NTGPEG
HTRF phospho-TBK1 (S172)	64TBKPEG
HTRF total-TBK1	64NTBPEG
HTRF phospho-IRF3 (S386)	6FRF3PEG
HTRF human IFNβ	62HIFNBPEG

Compounds	References
2'3' cGAMP	C161-Biolog
c-diAMP	C088-Biolog
c-diGMP	C057-Biolog
DMXAA	D5817 –Sigma-Aldrich

ORDERING INFORMATION

INNATE IMMUNITY

Sting Pathway

PRODUCT	500 TESTS	10.000 TESTS
Human STING binding kit	64BDSTGPEG	64BDSTGPEH
STING phospho-S366 kit	64STGPEG	64STGPEH
STING total kit	64NTGPEG	64NTGPEH
TBK1 phospho-S172 kit	64TBKPEG	64TBKPEH
TBK1 total kit	64NTBPEG	64NTBPEH
IRF3 phospho-S386 kit	6FRF3PEG	6FRF3PEH
NFκB phospho-S536 kit	64NFBPEG	64NFBPEH
NFκB total kit	64NFTPEG	64NFTPEH
Human IFN beta kit	62HIFNBPEG	62HIFNBPEH
Human TNF alpha kit	62HTNFAPEG	62HTNFAPEH
Human IL6 kit	62HIL06PEG	62HIL06PEH
Human IL1 beta kit	62HIL1BPEG	62HIL1BPEH

ADAPTIVE IMMUNITY

TCR Pathway

PRODUCT	500 TESTS	10.000 TESTS
ZAP-70 phospho-Y319 kit	64ZAPPEG	64ZAPPEH
ZAP-70 total kit	64ZATPEG	64ZATPEH
SLP-76 phospho S376 kit	63ADK076PEG	63ADK076PEH
SLP-76 total kit	63ADK077PEG	63ADK077PEH
SHP1 phospho-Y564 kit	64SH1PEG	64SH1PEH
SHP1 total kit	64NH1PEG	64NH1PEH
SHP2 phospho-Y542 kit	64SH2PEG	64SH2PEH
SHP2 total kit	64NH2PEG	64NH2PEH
AKT1 phospho-S473 kit	63ADK078PEG	63ADK078PEH
AKT1 total kit	63ADK079PEG	63ADK079PEH
AKT2 phospho S473 kit	63ADK080PEG	63ADK080PEH
AKT2 total kit	63ADK081PEG	63ADK081PEH
AKT3 phospho S473 kit	63ADK082PEG	63ADK082PEH
AKT3 total kit	63ADK083PEG	63ADK083PEH
AKT phospho-S473 kit*	64AKSPEG	64AKSPEH
AKT phospho-T308 kit	64AKTPEG	64AKTPEH
AKT total kit*	64NKTPEG	64NKTPEH
Advanced ERK phospho-T202/Y204 kit*	64AERPEG	64AERPEH
ERK total kit* -	64NRKPEG	64NRKPEH
Human IFN gamma kit*	62HIFNGPEG	62HIFNGPEH
Human IL2 kit	62HIL02PEG	62HIL02PEH

** kits also available under 1 x 96 tests*

Immune Checkpoints

PRODUCT	500 TESTS	10.000 TESTS
PD1/PD-L1 binding assay kit	64ICP01PEG	64ICP01PEH
CD47 / SIRP alpha binding assay kit	64ICP02PEG	64ICP02PEH
LAG3/MHC II binding assay kit	64ICP03PEG	64ICP03PEH
CTLA4/B7-1 binding assay kit	64ICP04PEG	64ICP04PEH
CTLA4/B7-2 binding assay kit	64ICP05PEG	64ICP05PEH

bulk sizes are available

FOR MORE INFORMATION
Europe and other countries +33(0)466 796 705 U.S. and Canada 1-888-963-4567 China +86 21 5018 9880
Japan +81-(0)43-306-8712 Visit www.cisbio.com to find a list of our regional distributors

cisbio
INTERACTION IS EVERYTHING