

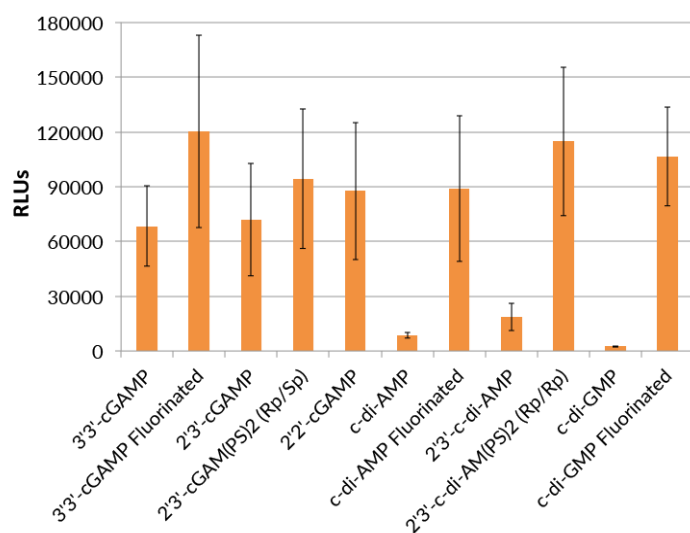
# Validation data for STING ligands

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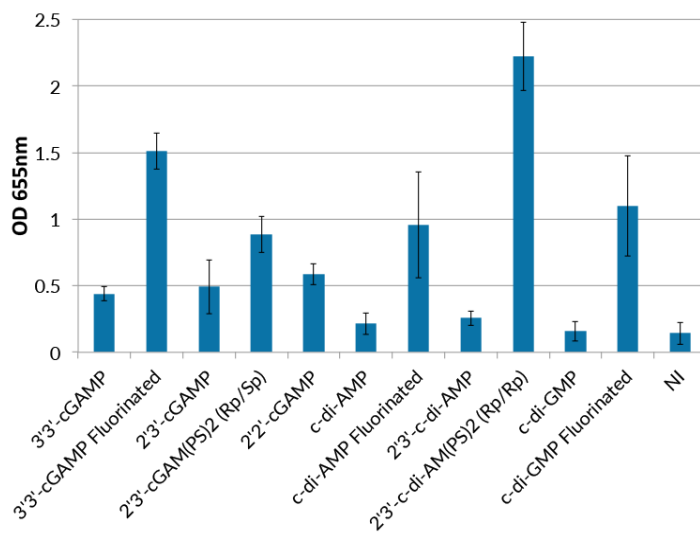
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Cyclic dinucleotides (CDNs) were recently found to bind to and activate STING, leading to a potent type I interferon (IFN) response. CDNs are important messengers in bacteria, affecting numerous responses of the prokaryotic cell, but also in mammalian cells, acting as agonists of the innate immune response. For your research needs, InvivoGen provides a large collection of high quality CDNs. They are chemically synthesized and are characterized by UHPLC, NMR and MS. For each lot, the biological activity is validated and the absence of bacterial contamination (e.g. endotoxins) is verified using cell-based assays. The activity of these CDNs has been compared (see figures 1 & 2) using the THP1-Dual™ cells, a cell line derived from the human THP-1 monocyte cell line by stable integration of two inducible reporter constructs. As a result, these cells allow the simultaneous study of the IFN regulatory factor (IRF) pathway, by assessing the activity of a secreted Lucia luciferase, and the NF-κB pathway, by monitoring the activity of SEAP.

## IRF INDUCTION (Lucia luciferase reporter)



## NF-κB INDUCTION (SEAP reporter)



THP1-Dual™ cells were stimulated for 24 hours with the STING ligands as shown (all at 10 μg/ml). The EC50 has been calculated for each ligand using the results obtained for the IRF induction (see table below).

**Figure 1:** IRF induction was determined by measuring the relative light units (RLUs) in a luminometer using QUANTI-Luc™, a Lucia luciferase detection reagent.

**Figure 2:** NF-κB induction was determined using QUANTI-Blue™, a SEAP detection reagent, and by reading the optical density (OD) at 655 nm. Non-induced cells (NI) have been included as a negative control.

EC50											
Ligand	3'3'-cGAMP	3'3'-cGAMP Fluorinated	2'3'-cGAMP	2'3'-cGAM(PS)2 (Rp/Sp)	2'2'-cGAMP	c-di-AMP	c-di-AMP Fluorinated	2'3'-c-di-AMP	2'3'-c-di-AM(PS)2 (Rp/Rp)	c-di-GMP	c-di-GMP Fluorinated
μM	12.0 ± 1.0	1.9 ± 0.4	9.3 ± 1.0	6.1 ± 0.7	6.4 ± 1.0	17.4 ± 2.3	5.6 ± 3.4	17.7 ± 2.1	2.1 ± 0.8	47.2 ± 6.5	3.4 ± 1.2
μg/ml	8.6 ± 0.7	1.4 ± 0.3	6.7 ± 0.7	4.5 ± 0.5	4.6 ± 0.7	12.2 ± 1.6	4.0 ± 2.4	12.4 ± 1.5	1.5 ± 0.6	34.7 ± 4.8	2.5 ± 0.9

### TECHNICAL SUPPORT

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