Validation data for STG-968

https://www.invivogen.com/sting-conjugatable-ligands

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Version 22G25-NJ

STG-968 is a "click-chemistry compatible" conjugatable STING ligand featuring an azido group added to the N-terminus of an analog of CL656, a well-known STING agonist. STG-968 efficiently triggers IRF- and NF-κB-mediated cellular responses **(Figure 1)**. STG-968 can be used to generate immune-stimulating antibody conjugates (ISACs) as conjugation to a Anti-HER2-hIgG1 and subsequent activation of STING has been validated using cellular assays. The Anti-HER2/STG-968 ISAC is more potent at inducing a STING-mediated response than STG-968 only in cells expressing HER2 **(Figure 2A)**. Of note, at high concentrations, STG-968, Anti-HER2/STG-968 ISAC and Anti-βGal/STG-968 ISAC, induce a STING-mediated cellular response, independently of HER2 expression **(Figure 2A and B)**. This observation could be explained by cellular uptake through endocytosis/pinocytosis.

Biological activity of STG-968

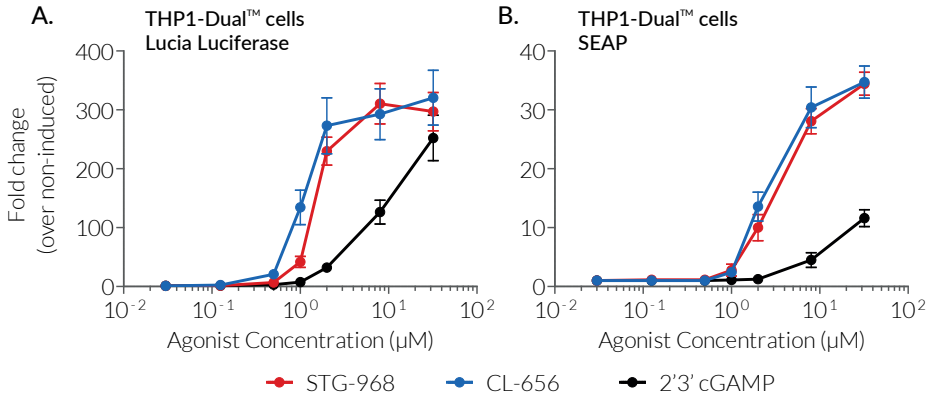

Figure 1: IRF and NF-KB responses induced by STING conjugatable ligand STG-968.
THP1-Dual™ cells were stimulated with increasing concentrations of STG-968, CL-656, or 2'3'-cGAMP. After overnight incubation, the IRF and NF-κB responses were determined by measuring Lucia luciferase and SEAP activity in the supernatant using QUANTI-Luc™ (A), or QUANTI-Blue™ Solution (B), respectively. Data are shown as a fold increase (mean ± SEM) over non-induced cells.

Biological activity of STG-968 conjugated to Anti-HER2-hIgG1

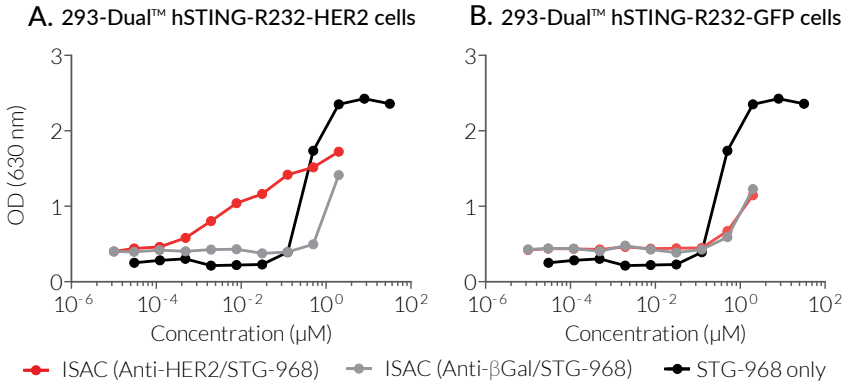

Figure 2: Dose-response of HER2-expressing STING reporter cells to Anti-HER2/STG-968 ISAC.
~ 5x10^5 293-Dual™ hSTING-R232-HER2 cells **(A)** or 293-Dual™ hSTING-R232-GFP control cells **(B)** were stimulated with increasing concentrations of Anti-HER2/STG-968 ISAC (Ratio 1:5), Anti-βGal/STG-968 ISAC (Ratio 1:5), or STG-968 only. After overnight incubation, the STING response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent. The optical density (OD) at 630 nm is shown.

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