

Validation data for 293-Dual™ hSTING H232 cells

<https://www.invivogen.com/293-dual-hsting-h232>

For research use only

Version 19K25-MM

293-Dual™ hSTING (ISG/KI-IFN β) H232 reporter cells were designed to study the H232 isoform of human STING. They stably express two inducible reporter constructs that enable the simultaneous analysis of interferon regulatory factor (IRF) activation, through monitoring the activity of SEAP, and interferon- β (IFN- β) expression, through assessing the activity of the secreted Lucia luciferase. These cells were stimulated with various cyclic dinucleotides (CDNs) and the murine STING ligand DMXAA. Stimulation with the mammalian (non-canonical) CDN 2'3'-cGAMP resulted in a dose-dependent IRF response (see figure 1). Modest IFN- β activity was observed in response to 3'3'-cGAMP Fluorinated and 2'3'-cGAM(PS)₂ (Rp/Sp), a bis-phosphorothioate analog of 2'3'-cGAMP (see figure 2). DMXAA and the bacterial (canonical) CDN 3'3'-cGAMP did not induce any detectable IRF or IFN- β induction in these cells.

IRF induction (SEAP reporter)

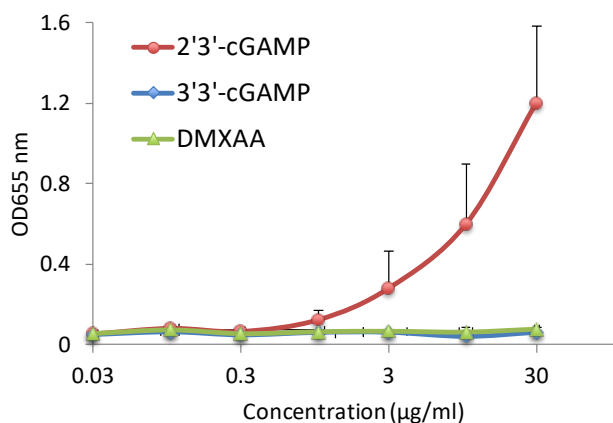


Figure 1: Dose-responses of 293-Dual™ hSTING H232 cells stimulated with 2'3'-cGAMP, 3'3'-cGAMP and DMXAA. After 24h incubation, IRF induction was assessed by measuring the levels of SEAP using QUANTI-Blue™ and by reading the optical density (OD) at 655 nm.

IFN- β induction (Lucia luciferase reporter)

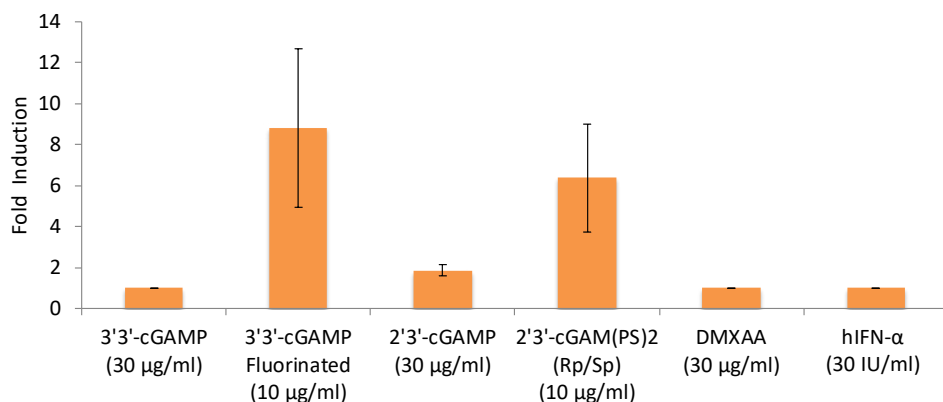


Figure 2: 293-Dual™ hSTING H232 cells were stimulated with 3'3'-cGAMP (30 µg/ml), 3'3'-cGAMP Fluorinated (10 µg/ml), 2'3'-cGAMP (30 µg/ml), 2'3'-cGAM(PS)₂ (Rp,Rp) (10 µg/ml), DMXAA (30 µg/ml) and human IFN- α (30 IU/ml). After 24h incubation, IFN- β induction was assessed by measuring the levels of Lucia luciferase using QUANTI-Luc™ and by reading the relative light units (RLUs) in a luminometer. The IFN- β response is expressed as a fold induction (calculated by dividing the RLUs for the treated cells by the RLUs for the untreated cells).

TECHNICAL SUPPORT

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