Validation data for THP1-Dual™ KI-hSTING-R232 cells

http://www.invivogen.com/thp1-dual-ki-hsting-r232

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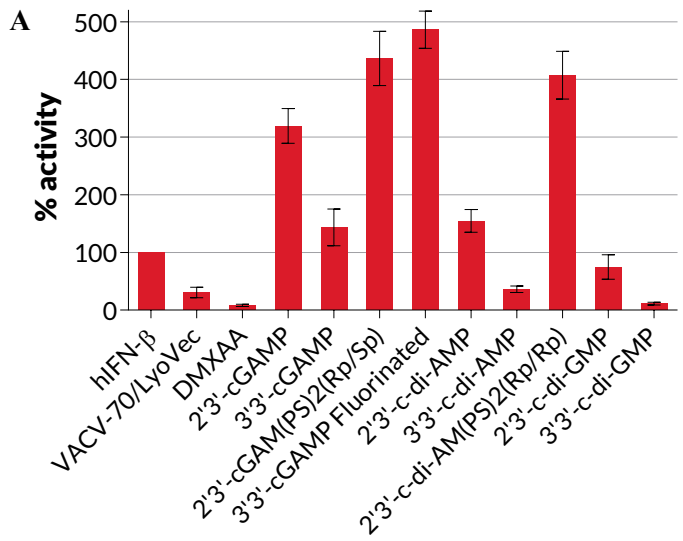
Version # 17F02-MM

THP1-Dual™ KI-hSTING-R232 cells are engineered monocytes that were specifically designed to monitor STING-mediated signaling. They were generated from THP1-Dual™ KO-STING (STING knockout) cells by stable knockin of the R232 human STING variant. These cell lines stably express two inducible reporter constructs that enable the simultaneous study of the NF-κB pathway, through monitoring the activity of SEAP, and the IRF pathway, through assessing the activity of the secreted Lucia luciferase. The IRF pathway (figure 1A) and NF-κB pathway (figure 1B) induction in THP1-Dual™ KI-hSTING-R232 cells in response to the STING ligands cyclic dinucleotides (CDNs) has been assessed. Indeed, CDNs induce a potent IRF and NF-κB response in these cells (see figure 1). As expected, these cells exhibit no detectable response to DMXAA (murine STING ligand). The knockin of the human STING variant (R232) in these cells has been confirmed by PCR and sequencing.

IRF INDUCTION (Lucia luciferase reporter)



NF-κB INDUCTION (SEAP reporter)

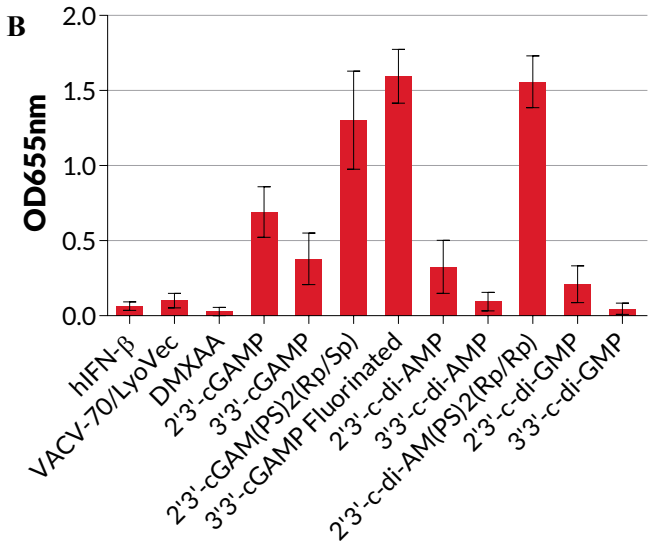


Figure 1: THP1-Dual™ KI-hSTING-R232 cells were stimulated with hIFN-β (1 x 10^4 U/ml), VACV-70 /LyoVec™ (1 µg/ml), DMXAA (100 µg/ml), 2'3'-cGAMP (30 µg/ml), 3'3'-cGAMP (30 µg/ml), 2'3'-cGAM(PS)₂ (Rp/SP) (30 µg/ml), 3'3'-cGAMP fluorinated (30 µg/ml), 2'3'-c-di-AMP (30 µg/ml), 3'3'-c-di-AMP (30 µg/ml), 2'3'-c-di-AM(PS)₂ (Rp/SP) (30 µg/ml), 2'3'-c-di-GMP (30 µg/ml) and 3'3'-c-di-GMP (30 µg/ml). **A)** After a 24h incubation, IRF activation was determined by measuring the relative light units (RLUs) in a luminometer using QUANTI-Luc™, a Lucia luciferase detection reagent. The IRF induction of each ligand is expressed relative to that of hIFN-β at 1x10^4 U/ml (taken as 100%). **B)** After a 24h incubation, NF-κB activation was determined using QUANTI-Blue™, a SEAP detection reagent, and by reading the optical density (OD) at 655 nm.

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