

# Validation data for THP1-Dual™ KI-hSTING-A162 cells

<http://www.invivogen.com/thp1-dual-ki-hsting-a162>

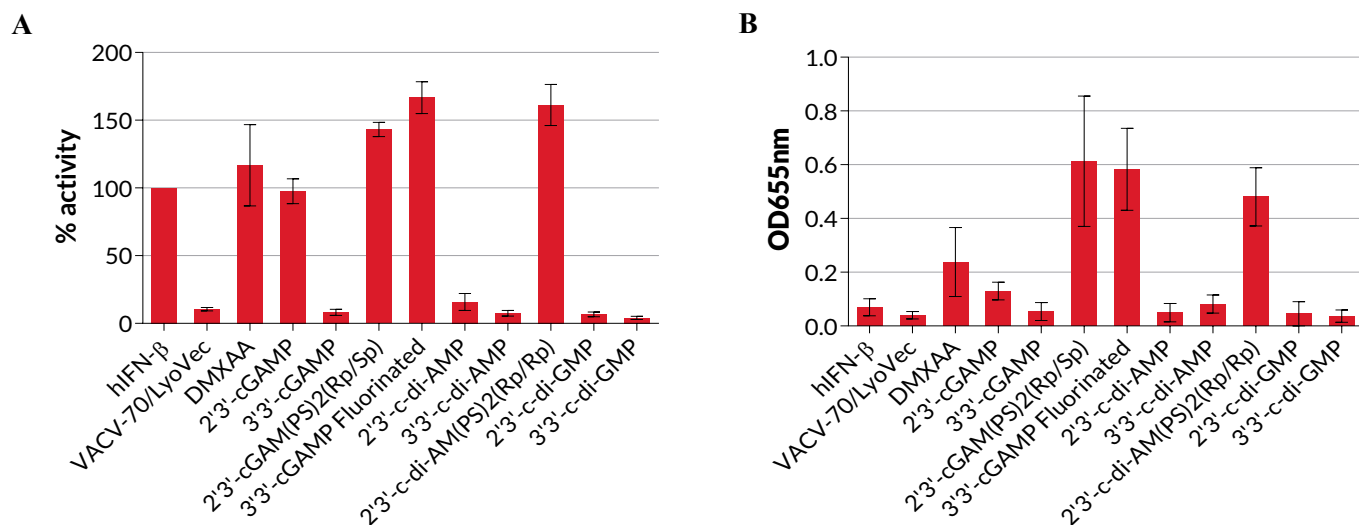
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THP1-Dual™ KI-hSTING-A162 cells are engineered monocytes that were specifically designed to monitor STING-mediated signaling. They were generated from THP1-Dual™ KO-STING (STING knockout) cells by stable knockin of the A162 human STING variant. These 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-A162 cells in response to the STING ligands cyclic dinucleotides (CDNs) has been assessed. As expected, they cells respond to CDNs including the murine STING ligand DMXAA (see figure 1). The knockin of the human STING variant (A162) 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-A162 cells were stimulated with hIFN-β (1 x 10<sup>4</sup> 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'-cGAMP(PS)<sub>2</sub> (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)<sub>2</sub> (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<sup>4</sup> 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.

### TECHNICAL SUPPORT

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