

Validation data for THP1-Dual™ KI-hSTING-H232 cells

<http://www.invivogen.com/thp1-dual-ki-hsting-h232>

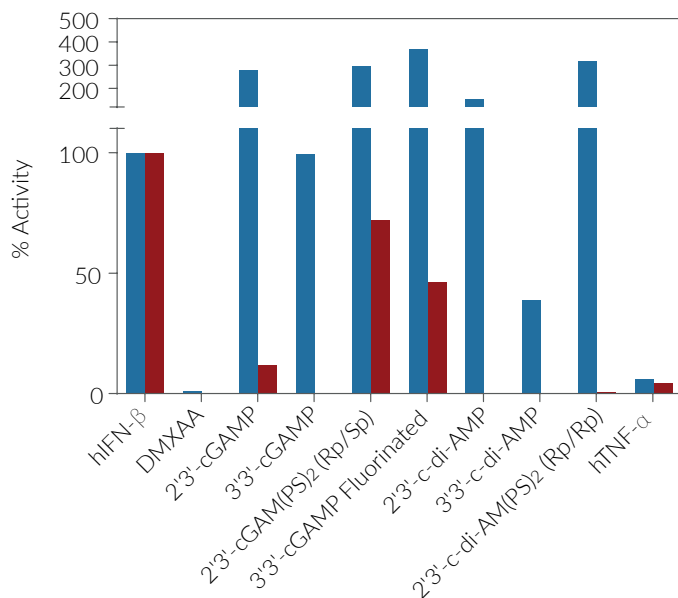
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Version 18E04-MM

THP1-Dual™ KI-hSTING-H232 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 knockin of the intronless coding sequence of R71-G230-**H232**-R293 human STING variant. They also stably express two inducible reporter constructs that enable the simultaneous study of the TBK1/IRF3 and NF-κB pathway through monitoring the activity of Lucia luciferase and SEAP respectively.

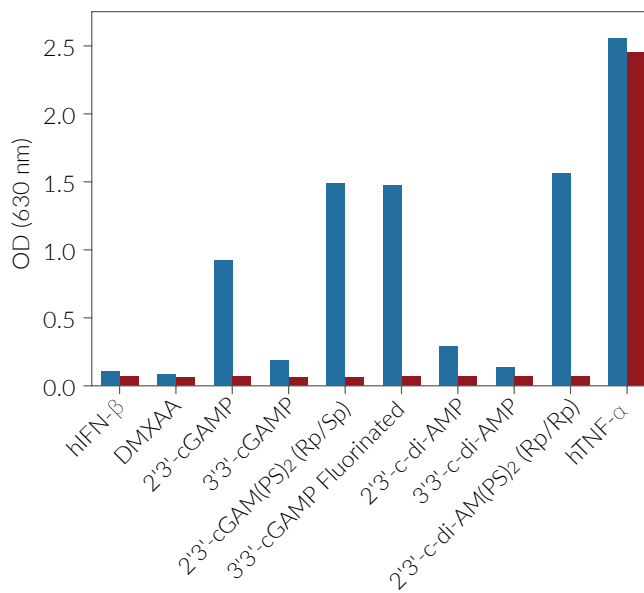
The IRF pathway (Figure A) and NF-κB pathway (Figure B) induction in THP1-Dual™ KI-hSTING-H232 cells in response to the STING ligands cyclic dinucleotides (CDNs) has been assessed. In contrast to THP1-Dual™ KI-hSTING-R232 cells expressing the “wild-type” R71-G230-**R232**-R293 human STING variant, THP1-Dual™ KI-hSTING-H232 cells do not respond to bacterial CDNs such as 3'3'-cGAMP and display only a weak IRF induction in response to mammalian CDNs such as 2'3'-cGAMP. Interestingly, fluorinated or bis-phosphorothioate analogs such as 3'3'-cGAMP Fluorinated or 2'3'-cGAM(PS)₂ (Rp/Sp) induce a moderate IRF induction. In THP1-Dual™ KI-hSTING-H232 cells, no NF-κB induction was observed following a 24-hour incubation with various CDNs.

A. IRF Induction (Lucia luciferase reporter)



■ THP1-Dual™ KI-hSTING-R232

B. NF-κB Induction (SEAP reporter)



■ THP1-Dual™ KI-hSTING-H232

Response profile of THP1-Dual™ KI-hSTING-H232 and THP1-Dual™ KI-hSTING-R232 cells. Cells were stimulated with hIFN-β (1 x 10⁴ U/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) and TNF-α (10 ng/ml). **A)** After a 24h incubation, IRF induction 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 1x10⁴ U/ml (taken as 100%). **B)** After a 24h incubation, NF-κB induction was determined using QUANTI-Blue™, a SEAP detection reagent, and by reading the optical density (OD) at 630 nm.

TECHNICAL SUPPORT

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