

# Validation data for THP1-Dual™ KI-hSTING-S154

<http://www.invivogen.com/thp1-dual-ki-hsting-s154>

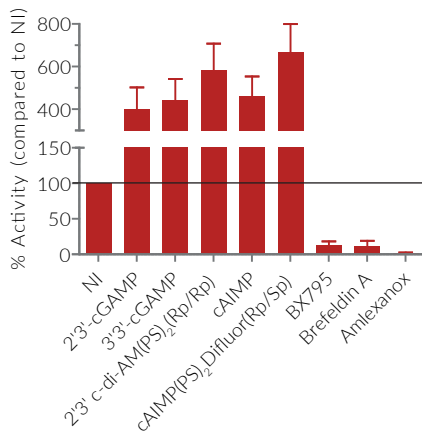
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Version # 17L19-NJ

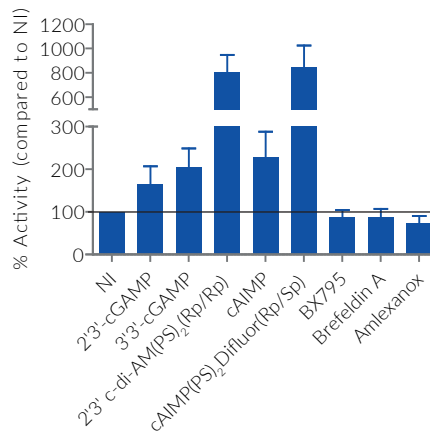
THP1-Dual™ KI-hSTING-S154 cells were generated from THP1-Dual™ KO-STING cells by knockin of the intronless coding sequence of the S154 hSTING (N154S) variant. The allele S154 contains a unique point mutation (N154S) in the “wild-type” R232 hSTING variant. This mutation confers gain-of-function and constitutive activation of STING and is associated with a chronic autoinflammatory disease, known as STING-associated vasculopathy with onset in infancy (SAVI). The THP1-Dual™ KI-hSTING-S154 cell line stably expresses 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 response of THP1-Dual™ KI-hSTING-S154 cells to cyclic dinucleotides is exacerbated compared to their basal constitutive activation (Figure 1a and 1b). Addition of TBK1 inhibitors (BX795, Amlexanox) or of an inhibitor of protein transport from ER to the Golgi apparatus (Brefeldin A) significantly impedes STING signaling via IRF (Figure 1a and Figure 2). These inhibitors have a lesser impact on NF-κB induction (Figure 1b). The THP1-Dual™ KI-hSTING-S154 cells are thus a useful reporter cell line for STING antagonist screening.

## Evaluation of IRF- and NF-κB-induced responses

### a. IRF induction (Lucia luciferase reporter)

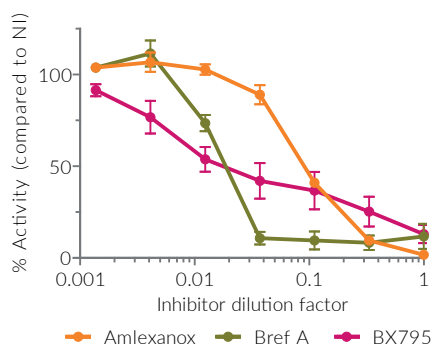


### b. NF-κB induction (SEAP reporter)



THP1-Dual™ KI-hSTING-S154 cells were stimulated with 10 µg/ml of various cyclic dinucleotides (2'3'-cGAMP, 3'3'-cGAMP, 2'3' c-di-AM(PS)<sub>2</sub>(Rp/Rp), cAIMP or cAIMP(PS)<sub>2</sub>Difluor(Rp/Sp), 30 µM BX795, 10 µM Brefeldin A, or 300 µg/ml Amlexanox. After overnight incubation, the IRF (panel a) and NF-κB (panel b) responses were determined using QUANTI-Luc™ and QUANTI-Blue™ respectively. Bars represent the % activity on the signal in non-induced (NI) cells.

## Evaluation of inhibitors on constitutive IRF-induced response



Dose inhibition of constitutively activated THP1-Dual™ KI-hSTING-S154 reporter cells with Amlexanox, Brefeldin A and BX795 inhibitors. Starting concentrations of Amlexanox (300 µg/ml), Brefeldin A (10 µM), or BX795 (30 µM) were diluted in 3-fold series. After overnight incubation, the ISG response was determined using QUANTI-Luc™. Data represent the % activity on the signal in non-induced (NI) cells without inhibitors.

### TECHNICAL SUPPORT

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