

Validation data for HEK-Blue™ ISG cells

<https://www.invivogen.com/hek-blue-isg>

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Version 18106-MM

HEK-Blue™ ISG cells were specifically designed to study the activation of the STING/TBK1/IRF3 signaling pathway by cyclic dinucleotides (CDNs). Biological activity has been assessed by measuring the levels of IRF-induced SEAP (secreted embryonic alkaline phosphatase) reporter activity (see figure 1). HEK-Blue™ ISG cells respond strongly to non-canonical CDNs namely 2'3'-cGAMP but do not respond to canonical CDNs such as 3'3'-cGAMP and its analog cAIMP. Interestingly, fluorinated or bis-phosphorothioate analogs such as 3'3'-cGAMP Fluorinated or 2'3'-c-di-AM(PS)₂ (Rp,Rp) induce a strong IRF induction. Of note, HEK-Blue™ ISG cells respond poorly to cytosolic DNA such as intracellular Poly(dA:dT). These cells display a robust response to human type I IFNs.

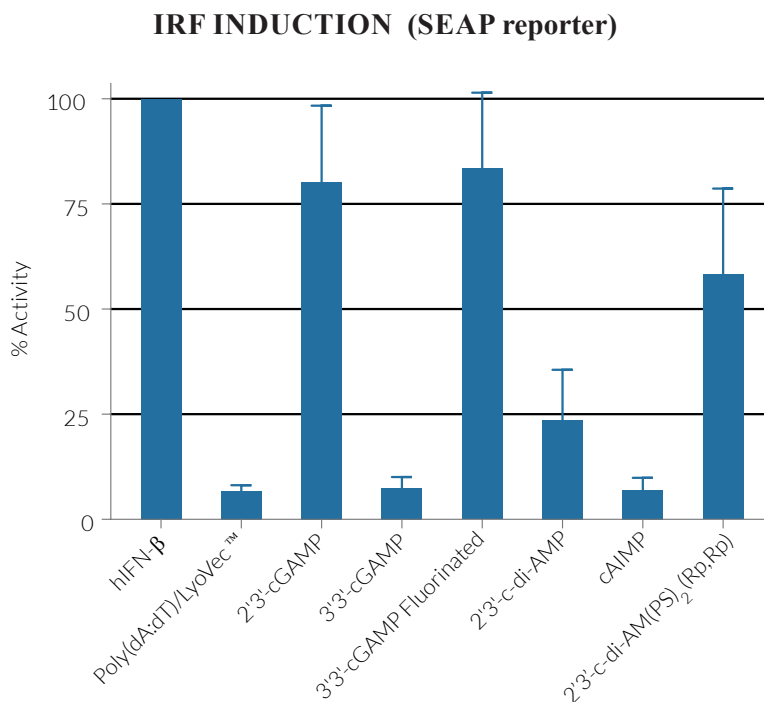


Figure 1. Response of HEK-Blue™ ISG cells to various CDNs, cytosolic dsDNA and IFN-β. HEK-Blue™ ISG cells were stimulated with 1×10^3 U/ml human IFN-β, 1 μg/ml poly(dA:dT)/LyoVec™, 30 μg/ml 2'3'-cGAMP, 3'3'-cGAMP, 3'3'-cGAMP Fluorinated, 2'3'-c-di-AMP, cAIMP, and 2'3'-c-di-AM(PS)₂ (Rp,Rp). After 24h incubation, IRF activation was determined using QUANTI-Blue™, a SEAP detection reagent, and by reading the optical density (OD) at 655 nm. The IRF induction of each ligand is expressed as % activity relative to that of human IFN-β at 1×10^3 U/ml (taken as 100%).

TECHNICAL SUPPORT

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