

# Validation data for 3p-hpRNA

<https://www.invivogen.com/3p-hprna>

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Version 23A19-MM

3p-hpRNA is a 5' triphosphate hairpin RNA that was generated by *in vitro* transcription of a sequence from influenza A virus. This 89-mer RNA oligonucleotide contains an uncapped 5'-triphosphate extremity and a double-strand fragment which are the structural features recognized by RIG-I. InvivoGen provides a collection of cell lines derived from the human lung A549 carcinoma, the mouse RAW macrophage and the human embryonic kidney HEK293 cells designed to facilitate the study of the RNA sensing RIG-I pathway. These cells are either knock-out for the RIG-I or MAVS gene (A549-derived or RAW-derived cells), or they overexpress the RIG-I gene (HEK293-derived cells).

Stimulation of A549-Dual™ and RAW-Lucia™ ISG cells with the RIG-I ligands, 5'ppp-dsRNA and 3p-hpRNA, complexed to LyoVec™ leads to a significant ISG response (Figures 1a and 2a). This response is much higher when using 3p-hpRNA compared to 5'ppp-dsRNA, especially in A549-Dual™ cells in which the maximal response is reached with 30 ng/ml of complexed 3p-hpRNA instead of 1 µg/ml for 5'ppp-dsRNA (Figure 1a). The NF-κB response to RIG-I ligands in A549-derived cells is similar to the ISG response although weaker (Figure 1b).

In RAW cells, which express low levels of RIG-I, the response to 3p-hpRNA is 6-fold higher compared to 5'ppp-dsRNA when used at 1 µg/ml. In contrast, in the RIG-I-KO cell lines this response is strongly diminished (Figure 2a). Stimulation of HEK-Lucia™ Null cells with RIG-I ligands results in a weak ISG response, whereas in HEK-Lucia™ RIG-I cells, this response is much stronger due to the constitutive overexpression of the RIG-I gene. Again, a higher ISG response is observed when using 3p-hpRNA versus 5'ppp-dsRNA (Figure 2b). Our data show that 3p-hpRNA is a potent and specific agonist of RIG-I.

## A549-Dual™ cellular response to 3p-hpRNA and 5'ppp-dsRNA

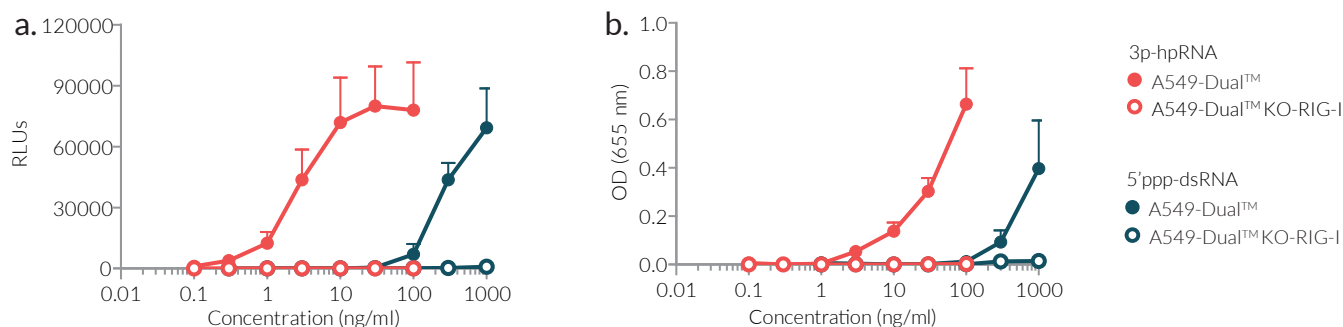


Figure 1: A549-Dual™ cell stimulation with 3p-hpRNA induces higher ISG and NF-κB responses than with 5'ppp-dsRNA.

Dose responses of A549-derived cells stimulated with 0.1-100 ng/ml 3p-hpRNA (complexed to LyoVec™) or 0.3 ng/ml to 1 µg/ml 5'ppp-dsRNA (complexed to LyoVec™). After overnight incubation, the ISG response was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™ 4 Lucia/Gaussia and expressed as relative light units (RLUs) (a), and the NF-κB response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent, and by reading the optical density (OD) at 655 nm (b).

## RAW-Lucia™ ISG and HEK-Lucia™ RIG-I cellular response to 3p-hpRNA and 5'ppp-dsRNA

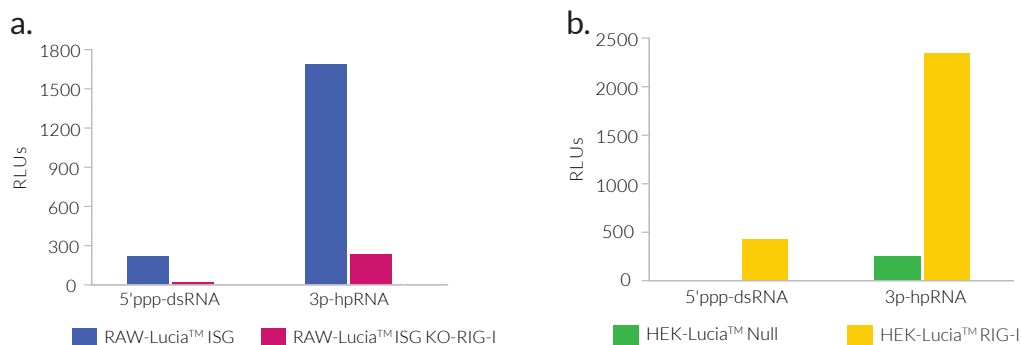


Figure 2: RAW-Lucia™ and HEK-Lucia™ RIG-I cell stimulation with 3p-hpRNA induces a better ISG response than with 5'ppp-dsRNA.

RAW-derived (a) and HEK-derived (b) cells were stimulated with 1 µg/ml 5'ppp-dsRNA (complexed to LyoVec™) or 3p-hpRNA (complexed to LyoVec™). After overnight incubation, the ISG response was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™ 4 Lucia/Gaussia and expressed as relative light units (RLUs).

### TECHNICAL SUPPORT

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