

# Validation data for 3p-hpRNA/LyoVec™

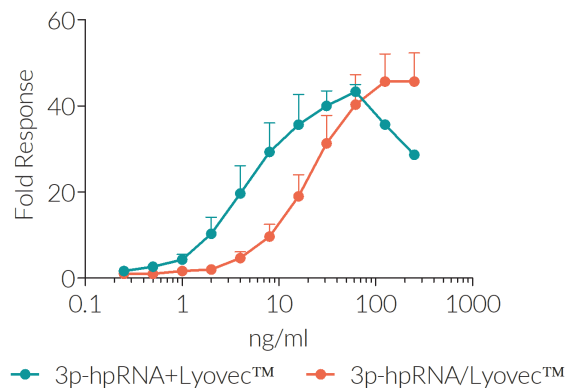
<http://www.invivogen.com/3p-hpRNA>

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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, the founding member of the RIG-I like receptor (RLR) family. 3p-hpRNA must be delivered to the cytoplasm, for example by using a transfection agent, such as the cationic lipid LyoVec™. To facilitate your research, InvivoGen provides pre-complexed 3p-hpRNA/LyoVec™. The potency of RIG-I agonists can be assessed using the A549-Dual™ cells which are derived from the human lung A549 carcinoma. These cells express two reporter genes: an ISG (interferon stimulated gene)-inducible Lucia luciferase, and an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase). Stimulation of A549-Dual™ cells with 3p-hpRNA/LyoVec™ leads to a significant ISG response. Note that freshly prepared complexes are ~10-times more potent than pre-formed complexes for RIG-I induction using our cellular assay (Figure 1).

## 3p-hpRNA-induced RIG-I signaling in A549-Dual™ cells



**Figure 1: A549-Dual™ cell stimulation with 3p-hpRNA.** Dose responses of A549-derived cells stimulated with pre-complexed 3p-hpRNA/LyoVec™ (red) or freshly prepared 3p-hpRNA+LyoVec™ (blue). After overnight incubation, the ISG response was determined by assessing the Lucia luciferase activity in the supernatant using QUANTI-Luc™ detection reagent. Fold increase in response over non-induced cells is shown as mean+sem.

### TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873  
InvivoGen USA (International): +1 (858) 457-5873  
InvivoGen Europe: +33 (0) 5-62-71-69-39  
InvivoGen Hong Kong: +852 3-622-34-80  
E-mail: [info@invivogen.com](mailto:info@invivogen.com)

 **InvivoGen**  
[www.invivogen.com](http://www.invivogen.com)