Validation data for G3-YSD Control

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Version 24F17-MM

G3-YSD Control is a negative control for G3-YSD, a potent cGAS (cyclic GMP-AMP synthase, cGAMP synthase) agonist. This negative control differs from the G3-YSD agonist in the hairpin-flanking nucleoside trimers. While G3-YSD is flanked with guanosine trimers (G3) conferring its agonist activity, G3-YSD Control is flanked with cytidine trimers (C3) abrogating cGAS activation. The activity of this ligand has been tested using THP1-Dual™ cells which express multiple cytosolic DNA sensors (CDSs) and two inducible reporter genes (interferon regulatory factor (IRF)-inducible secreted Lucia luciferase and NF-κB-inducible SEAP). Stimulation of these cells with G3-YSD complexed to LyoVec™ leads to a significant IRF response. As expected, intracellular delivery of G3-YSD Control does not induce an IRF response in these cells (**Figure 1**).

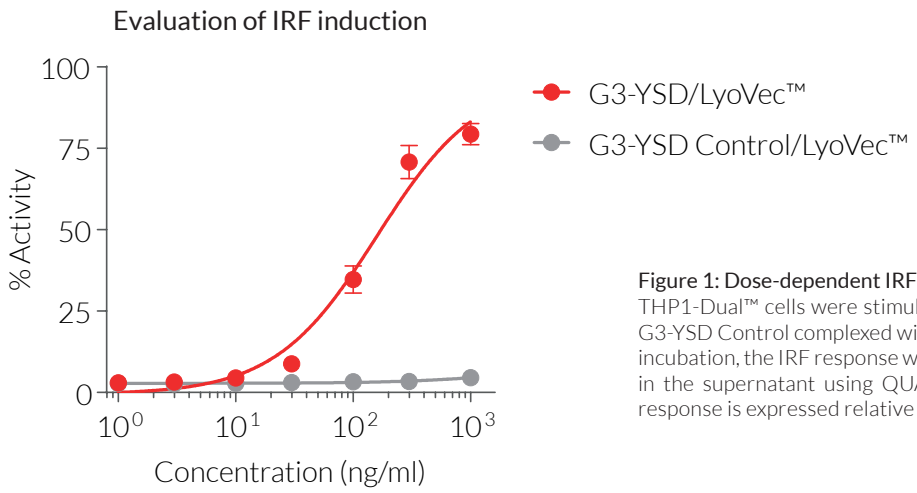

Figure 1: Dose-dependent IRF response in THP1-Dual™ cells.
THP1-Dual™ cells were stimulated with increasing concentrations of G3-YSD or G3-YSD Control complexed with LyoVec™, or 1 x 10^4 U/ml hIFN-β. After overnight incubation, the IRF response was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™ 4 Lucia/Gaussia. For each ligand, the response is expressed relative to that of hIFN-β (taken as 100%).

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