Validation data for G3-YSD

http://www.invivogen.com/g3-ysd

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Version 18E18-MM

G3-YSD (G3-ended Y-form Short DNA) is a potent agonist for the cytosolic DNA sensor cGAS. This 26-mer DNA palindromic sequence derived from HIV-1 contains unpaired guanosines trimers (G3) in Y-form dsDNA-ssDNA junctions. These guanosine-overhangs have been identified as minimal recognition motifs for cGAS. The activity of this ligand has been tested using the 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 the CDS ligands, G3-YSD and VACV-70, complexed to LyoVec™ leads to a significant IRF response (Figure 1). Of note, this response is stronger when using G3-YSD compared to VACV-70. As expected, intracellular delivery of G3-YSD Control does not induce an IRF response in these cells (Figure 1). Importantly, the IRF response to intracellular delivery of G3-YSD is strictly cGAS-dependent (Figure 2).

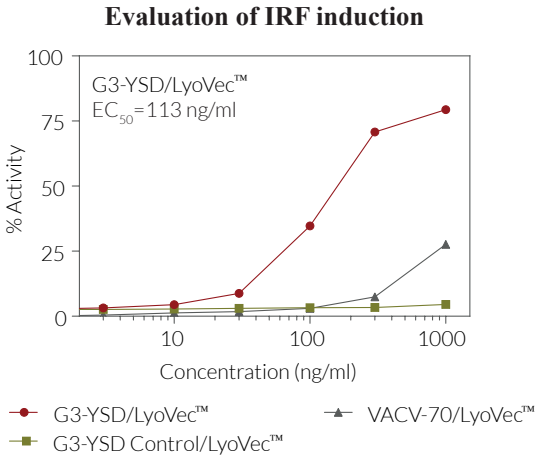

Figure 1: Intracellular delivery of G3-YSD induces a potent IRF response in a dose-dependent manner.
THP1-Dual™ cells were stimulated with increasing concentrations of G3-YSD, G3-YSD Control or VACV-70 complexed with LyoVec™, or 1 x 10^4 U/ml hIFN-β. After overnight incubation, the IRF response was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™. For each ligand, the response is expressed relative to that of hIFN-β (taken as 100%).

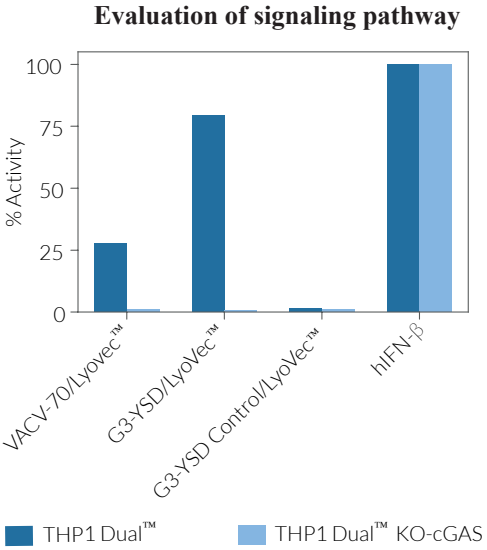

Figure 2: IRF response upon intracellular delivery of G3-YSD is strictly dependent on cGAS.
THP1-Dual™ and THP1-Dual™ KO-cGAS cells were stimulated with 1 µg/ml G3-YSD, G3-YSD Control or VACV-70 complexed with LyoVec™, or 1 x 10^4 U/ml hIFN-β. After overnight incubation, the IRF response was assessed using QUANTI-Luc™ and expressed relative to that of hIFN-β (taken as 100%).

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