Validation data for THP1-Dual™ cells

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Version 20B26-MM

THP1-Dual[™] cells are derived from the human THP-1 monocyte cell line by stable integration of two inducible secreted reporter genes: Lucia luciferase and SEAP (secreted embryonic alkaline phosphatase). As a result, they allow the simultaneous study of the NF-κB pathway, by monitoring the activity of SEAP, and the IRF (interferon regulatory factor) pathway, by assessing the activity of Lucia luciferase. THP1-Dual[™] cells induce activation of NF-κB in response to certain TLR agonists, such as Pam3CSK4 and flagellin. They trigger the IRF pathway upon stimulation with type I interferons (IFNs) and RLR (RIG-I-like receptor), CDS (cytosolic dsDNA sensor) or STING agonists, such as transfected poly(I:C) or poly(dA:dT) and 2'3'cGAMP respectively.

NF-κB and IRF responses of THP1-Dual[™] Cells to PRR ligands

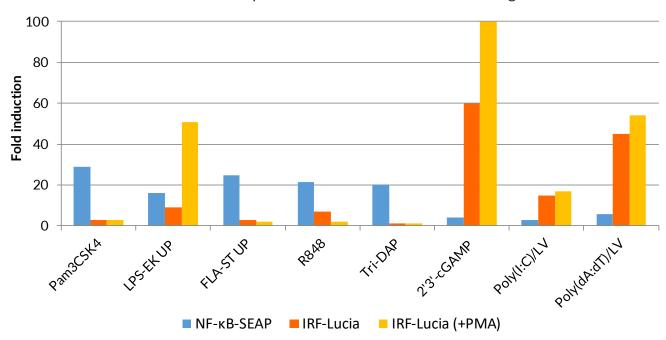


Figure 1: NF-kB/IRF dual response of THP1-Dual cells. Cells were pretreated or not pretreated with PMA (Phorbol 12-myristate 13-acetate; 20 ng/ml for 3 hours) and stimulated with 1 ng/ml Pam3CSK4 (TLR2), 100 ng/ml LPS-EK UP (TLR4), 100 ng/ml FLA-ST UP (TLR5), 10 μg/ml R848 (TLR7/8), 10 μg/ml Tri-DAP (NOD1), 3 μg/ml 2'3'-cGAMP (STING), 1 μg/ml poly(l:C)/LyoVec (RLR) or 100 ng/ml poly(dA:dT)/LyoVec (CDS). After 24h incubation, NF-κB and IRF activation were assessed by measuring the levels of SEAP and Lucia luciferase using QUANTI-Blue and QUANTI-Luc, respectively. With QUANTI-Blue the levels of SEAP were determined by reading the optical density (OD) at 655 nm. With QUANTI-Luc the levels of Lucia luciferase were determined by measuring the relative light units (RLUs) in a luminometer.

