Validation data for J774-Dual™ cells

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J774-Dual™ cells have been derived from the mouse J774.1 macrophage-like cell line by stable integration of two inducible reporter constructs. J774-Dual™ cells express an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene and an interferon regulatory factor (IRF)-inducible Lucia luciferase gene. Thus, J774-Dual™ cells allow to simultaneously study the NF-κB pathway, by assessing the activity of SEAP, and the IRF pathway, by monitoring the activity of Lucia luciferase.

**NF-κB Response of J774-Dual cells to PRR ligands**

![NF-κB Response Graph]

**Figure 1:** J774-Dual™ cells were stimulated with various PRR agonists known to activate the NF-κB pathway: Pam3CSK4 (TLR1/2 ligand, 1 μg/ml), ultrapure lipopolysaccharide from *Escherichia coli* K12 (LPS-EK UP, TLR4 ligand, 100 ng/ml), poly(I:C) (TLR3 ligand, 10 ng/ml), ODN1826 (TLR9 ligand, 10 μg/ml) and L18-MDP (NOD2 ligand, 10 μg/ml) NF-κB-induced SEAP activity was assessed using QUANTI-Blue™, a SEAP detection reagent, and by reading the optical density (OD) at 655 nm.

**IRF Response of J774-Dual cells to various PRR ligands**

![IRF Response Graph]

**Figure 2:** J774-Dual™ cells were stimulated with various PRR agonists known to activate the IRF pathway: 2’3’-cGAMP (STING ligand, 2 μg/ml), poly(I:C) complexed with the transfection reagent LyoVec™ (LV) (RIG-I/MDA-5 ligand, 30 ng/ml), and 3’ppp-dsRNA (3P-RNA) complexed with LyoVec™ (RIG-I ligand, 1 μg/ml). After 24h, IRF-induced Lucia luciferase activity was determined by measuring relative light units (RLUs) using the QUANTI-Luc™ assay and a luminometer.