

Validation data for HEK-Blue™ TNF- α cells

For research use only

Version # 15K19-MM

HEK-Blue™ TNF- α cells are engineered HEK293 cells that stably express an NF- κ B- and AP-1-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. These cells were rendered unresponsive to IL-1 β by knocking-out the MyD88 gene. Stimulation of HEK-Blue™ TNF- α cells with human and murine TNF- α triggers the activation of the NF- κ B/AP-1-inducible promoter and the production of SEAP. They do not respond to other cytokines such as IL-1 β (see figure 1).

Response of HEK-Blue™ TNF- α cells to TNF- α and IL-1 β

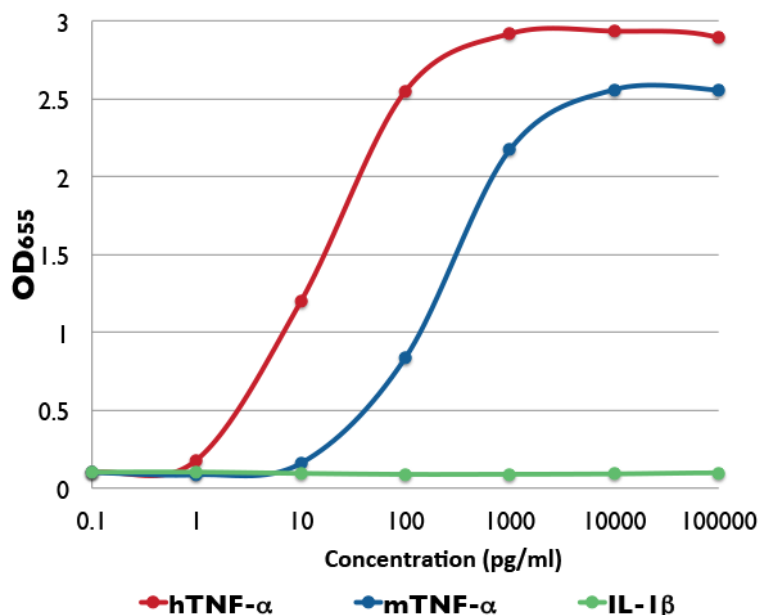


Figure 1: Response of HEK-Blue™ TNF- α cells to TNF- α and IL-1 β . Cells were incubated with increasing concentrations of human and murine TNF- α and human IL-1 β . After a 24h incubation, NF- κ B/AP-1 activation was assessed by measuring the levels of SEAP in the supernatant using QUANTI-Blue™ Solution, a SEAP detection reagent, and by reading the optical density (OD) at 655 nm.

Ligand	EC50 (cytokines diluted in medium)	EC50 (cytokines diluted in water)	Response ratio
Human TNF- α	0.01 ng/ml	0.7 ng/ml	30
Murine TNF- α	0.1 ng/ml	3 ng/ml	25

The EC50 values were determined using commercially available cytokines diluted in cell culture medium (DMEM containing 10% fetal calf serum) or water. The response ratio was calculated by dividing the OD at 655 nm for the treated cells by the OD at 655 nm for the untreated cells.

TECHNICAL SUPPORT

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