# Validation data for HEK-Blue<sup>™</sup> CD40L cells

https://www.invivogen.com/hek-blue-cd40l

## For research use only

Version 23J17-AK

HEK-Blue<sup>TM</sup> CD40L cells allow the detection of bioactive CD40L (CD40 Ligand) by monitoring the activation of the NF- $\kappa$ B pathway. These cells were generated by the stable transfection of HEK293 cells with the human CD40 as well as SEAP reporter gene to obtain a fully active CD40-dependent signaling pathway. These cells respond strongly to human and murine CD40L (Figures 1 and 2). These cells can also be used to screen for molecules that inhibit CD40L signaling by using an anti-hCD40L-lgG anitbody (Figure 3).

# Dose-response to human CD40L 3.0 2.5 2.5 2.0 0.5 0.0 10-3 10-1 101 103 hCD40L (ng/ml)

Figure 1. Dose-response of HEK-Blue™ CD40L cells to recombinant CD40L. Cells were stimulated with increasing concentrations of recombinant human (h)CD40L. After overnight incubation, the response was determined using QUANTI-Blue™, a SEAP detection reagent. The optical density (OD) at 650 nm is shown as mean ± SEM.

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Figure 2. Response of HEK-Blue<sup>TM</sup> CD40L cells to a panel of cytokines. Cells were stimulated with various human recombinant cytokines: hIFN- $\alpha$ 2b or hIFN- $\beta$ -1a (1000 U/ml), hIFN- $\gamma$  (1 µg/ml), hIL-1 $\beta$  (1 ng/ml), hIL-4 (100 ng/ml), hIL-6 (100 ng/ml), hIL-13 (100 ng/ml), hIL-18 (100 ng/ml), hTNF- $\alpha$  (1 ng/ml), hTGF- $\beta$ 1 (100 ng/ml), hCD40L (100 ng/ml), and mCD40L (100 ng/ml). After overnight incubation, SEAP activity was assessed using QUANTI-Blue<sup>TM</sup>. The OD at 650 nm is shown as mean  $\pm$  SEM.

### Neutralization of hCD40L response using Anti-hCD40L-IgG mAb

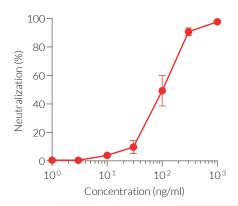


Figure 3. Dose-dependent inhibition of HEK-Blue™ CD40L cell response using Anti-hCD40L-lgG mAb. A serial dilution of Anti-hCD40L-hlgG monoclonal antibody (mAb) was incubated with 100 ng/ml of recombinant human CD40L for 30 minutes prior to the addition of the HEK-Blue™ CD40L cells. After overnight incubation, the SEAP response was determined using QUANTI-Blue™, a SEAP detection reagent. Data are presented as percentage of neutralization (mean ± SEM).

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