Validation data for HEK-Blue[™] IL-17C cells

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Version 19H13-MM

HEK-Blue[™] IL-17C cells allow the detection of bioactive human (hIL-17C) and murine IL-17C (mIL-17C) by monitoring NF- κ B and AP-1 activation. They were generated by stable transfection of HEK293 cells with the genes encoding the IL-17C heterodimeric receptor (IL-17RA/IL-17RE), the Act1 adaptor molecule, as well as an NF- κ B- and AP-1-inducible SEAP (secreted embryonic alkaline phosphatase) reporter. These cells display high sensitivity to h/mIL-17C and h/mIL-17E with moderate sensitivity to hIL-17A (**figures 1 & 2**). They do not respond to mIL-17A nor h/mIL-17F. Of note, as HEK293 cells endogenously express the TNF- α and IL-1 β receptors, these cells respond to TNF- α and IL-1 β (**figure 2**). Additionally, these cells can be used for the screening of anti-IL-17C antibodies (**figure 3**).

Evaluation of IL-17 response



| Cytokine | EC ₅₀ (ng/ml) |
|-----------|--------------------------|
| hIL-17C 🔸 | 11.5 ± 6.4 |
| mIL-17C 🔶 | 6.8 ± 4.4 |
| hIL-17E 🔸 | 1.3 ± 1 |
| mIL-17E 🔸 | 1.2 ± 1 |
| hIL-17A 🔸 | 24 ± 13 |
| mILI7-A 🔸 | N/A |

Figure 1. Dose-response of HEK-Blue[™] IL-17C cells to recombinant IL-17 cytokines. Cells were stimulated with increasing concentrations of recombinant human or murine IL-17C, IL-17E, and IL-17A. After overnight incubation, the NF- κ B response was determined using QUANTI-Blue[™] Solution, a SEAP detection reagent, and reading the optical density (OD) at 630 nm. EC₅₀ values are shown as mean ± SD.



Figure 2. Cytokine response profile of HEK-Blue[™] IL-17C cells. Cells were stimulated with various human and murine recombinant cytokines: 10 ng/ml of hIL-17C, mIL-17C, hIL-17E, mIL-17E, hIL-17A, mIL-17A, 1x10⁴ IU/ml of hIFN- α , hIFN- β , 100 ng/ml of hIFN- γ , and 1 ng/ml of hIL-1 β and hTNF- α . After overnight incubation, SEAP activity was assessed using QUANTI-Blue[™] Solution. HEK-Blue[™] IL-17C cells do not respond to hIL-17B nor hIL-17D (data not shown).

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Evaluation of IL-17 signaling inhibition



Figure 3. Dose-dependent inhibition of the human IL-17C response using a neutralizing antibody against the human IL-17RA receptor in HEK-Blue[™] IL-17C cells. The antibody was incubated with HEK-Blue[™] IL-17C cells for 30 minutes prior to the addition of 30 ng/ml of hIL-17C. After overnight incubation, SEAP activity in the cell culture supernatant was assessed using QUANTI-Blue[™] Solution. Data represent % inhibition of reporter activity.

