Validation data for HEK-Blue™ hACE2-TMPRSS2 cells

https://www.invivogen.com/hek-blue-hace2-tmprss2-cells

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Version 21F10-ED

HEK-Blue™ hACE2-TMPRSS2 cells were generated from the HEK-Blue™ hACE2 cells, which derive from the human embryonic kidney 293 (HEK-293) cell line. Overexpression of the SARS-CoV-2 host receptors ACE2 and TMPRSS2 in HEK-Blue™ hACE2-TMPRSS2 cells has been verified by RT-qPCR **(Figure 1)** and flow cytometry **(Figure 2)**. In comparison to their parental cell line, these cells display increased sensitivity to pseudotyped lentiviral particles expressing the SARS-CoV-2 Spike protein **(Figure 3).** Additionally, since HEK-Blue™ hACE2-TMPRSS2 cells express an NF-κB-dependent SEAP reporter, they can be used as 'acceptor' cells in InvivoGen's Spike-ACE2-dependent cell fusion assay **(Figure 4).**

Validation of *ACE2* and TMPRSS2 overexpression by RT-qPCR and flow cytometry

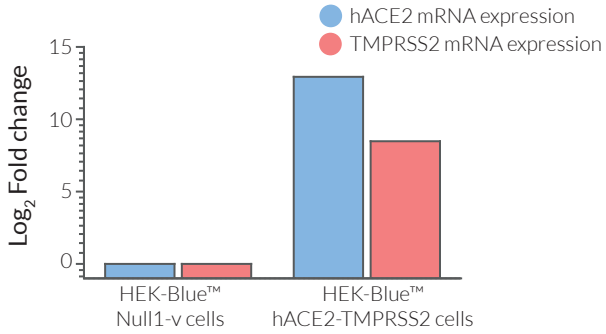

Figure 1: hACE2 and TMPRSS2 mRNA expression. Total mRNA was extracted from ~5x10^5 HEK-Blue™Null1-v and HEK-Blue™ hACE2-TMPRSS2 cells. ACE2 and TMPRSS2 mRNA were amplified using quantitative RT-qPCR. Data are represented as the log2 fold change comparing hACE2 or TMPRSS2 relative expression between the cell lines.

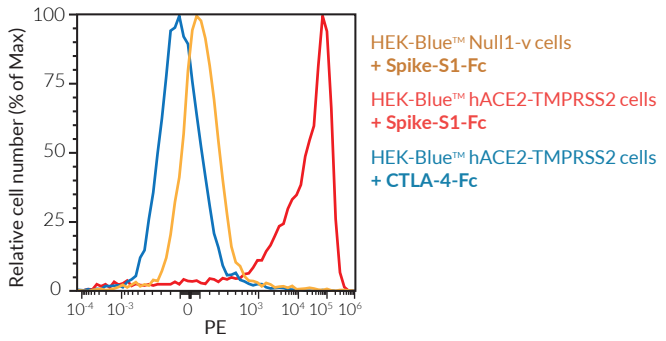

Figure 2: Surface expression of hACE2. ~5x10^5 HEK-Blue™ Null1-v and HEK-Blue™ hACE2-TMPRSS2 cells were incubated with 1 µg of Spike-S1-Fc or CTLA-4-Fc fusion proteins for 1 hr at 4°C. Cells were then washed and incubated with 0.5 µg of a goat anti-hIgG1-Fc antibody coupled to PE for 1 hr at 4°C. Cell surface staining was analyzed by flow cytometry.

Infection by SARS-CoV-2 Spike pseudotyped lentiviral particles

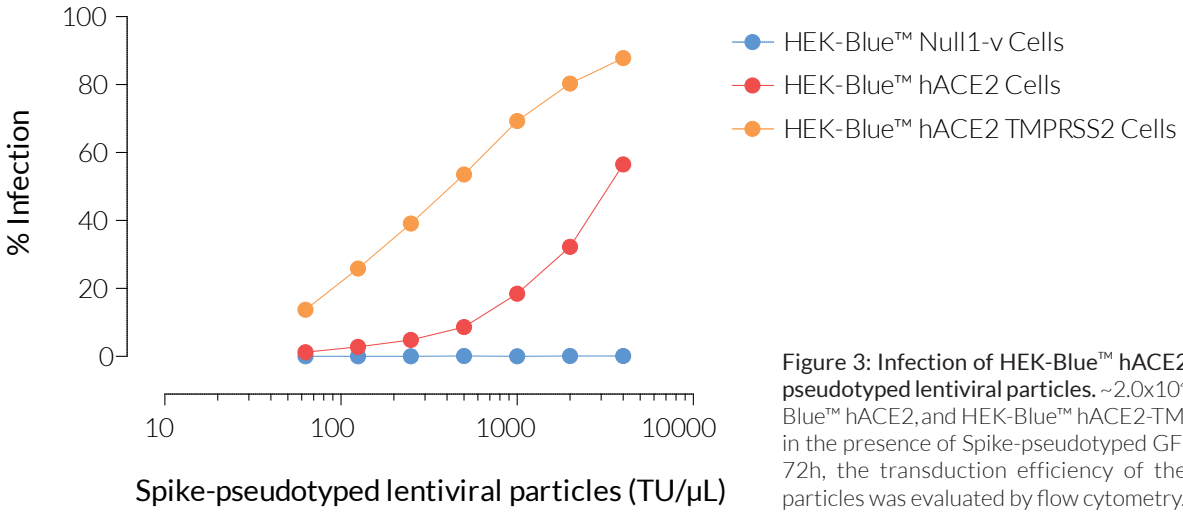

Figure 3: Infection of HEK-Blue™ hACE2-TMPRSS2 cells by Spike pseudotyped lentiviral particles. ~2.0x10^4 HEK-Blue™ Null1-v, HEK-Blue™ hACE2, and HEK-Blue™ hACE2-TMPRSS2 cells were cultured in the presence of Spike-pseudotyped GFP lentiviral particles. After 72h, the transduction efficiency of the Spike peudotyped GFP particles was evaluated by flow cytometry.

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Assessing cell fusion with InvivoGen's COVID-19 cell lines

To generate the 'donor cell line', 293-hMyD88 cells were transiently transfected with InvivoGen's pUNO1-Spike expression plasmid, which encodes the Wuhan-Hu-1 Spike with a functional furin cleavage site to faciliate cell fusion. Upon co-culture of a dilution series of the 'donor cell line' with HEK-Blue™ hACE2-TMPRSS2 cells ('acceptor' cells), fusion was triggered. Following this, MyD88 activated a signalling cascade in the HEK-Blue™ hACE2-TMPRSS2 cells, ultimately leading to readily assessable SEAP production.

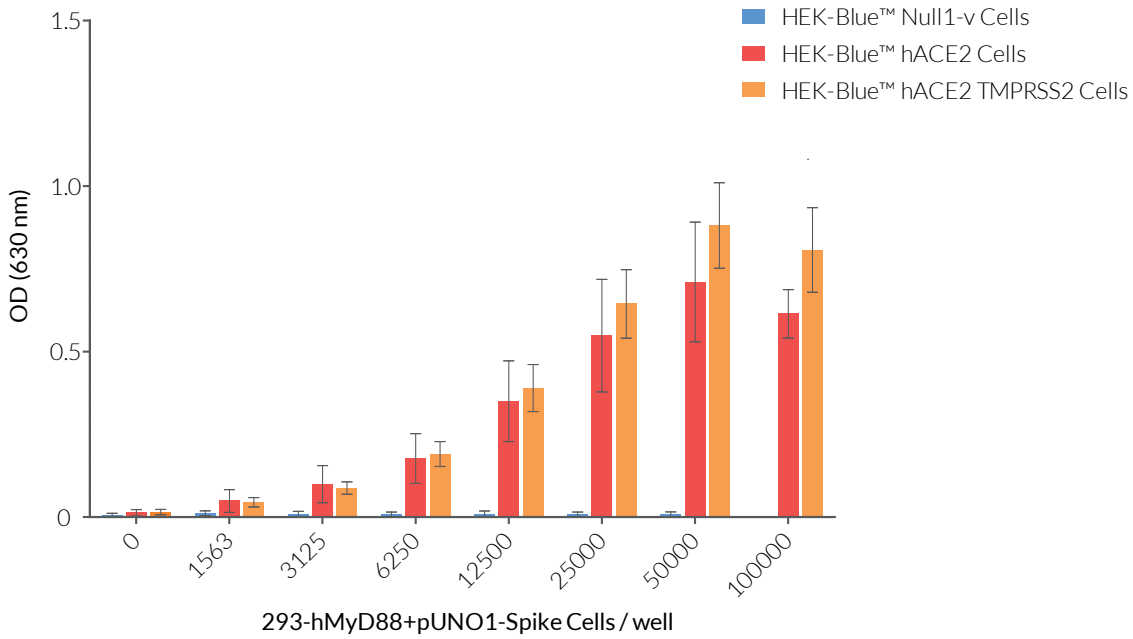

Figure 4: Assessing cell fusion with 293-hMyD88 cells. 293-hMyD88 cells were transiently transfected with a SARS-CoV-2 Spike expression plasmid (pUNO1-Spike) using LyoVec™. After 24 hours, the cells were washed, and a dilution series of the 'donor' 293-hMyD88-Spike cells was co-cultured with either 2.0 x10^4 HEK-Blue™ Null1-v, HEK-Blue™ hACE2, or HEK-Blue™ hACE2-TMPRSS2 cells. After overnight incubation, cell fusion was assessed by measuring the activity of SEAP in the supernatant using QUANTI-Blue™ Solution, a SEAP detection reagent. Data are presented as OD630nm ± SEM.