

Validation data for A549-hACE2 cells

<https://www.invivogen.com/a549-hace2tmprss2-cells>

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A549-hACE2 cells were generated from the A549 lung carcinoma cell line. The A549 cell line is a well-characterized cellular model of the alveolar basal epithelial lung cells for the study of respiratory infections. ACE2 overexpression in A549-hACE2 cells has been verified by RT-qPCR (Figure 1), and cell surface staining (Figure 2). Unlike their parental cell line, A549-hACE2 cells can be infected with pseudotyped lentiviral particles expressing the SARS-CoV-2 Spike protein (Figure 3, and data not shown). As expected, lentiviral particles expressing the globally dominant Spike G614-variant have greater infectivity of ACE2-expressing target cells, when compared to particles expressing the original D614 Spike protein (Figure 3, bottom row).

Validation of ACE2 overexpression by RT-qPCR

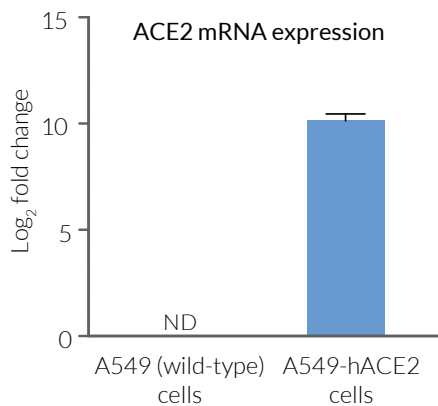


Figure 1: Human ACE2 mRNA expression in A549-hACE2 cells. Total mRNA was extracted from $\sim 1 \times 10^6$ A549 (wild-type) and A549-hACE2 cells. ACE2 mRNA was amplified using quantitative RT-qPCR. Data are represented as the log₂ fold change comparing hACE2 expression to a housekeeping gene. ND: not detected.

Validation of ACE2 surface expression by FACS

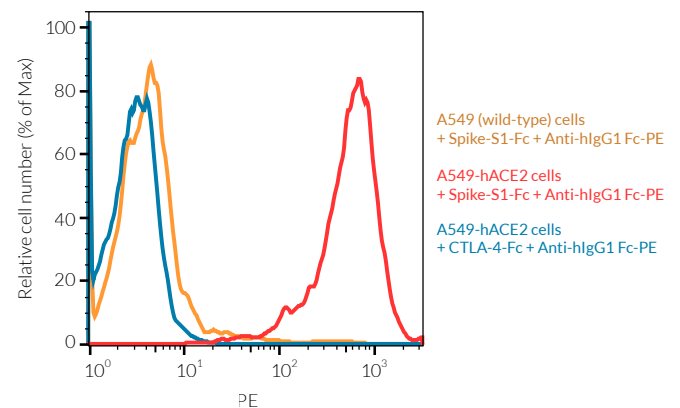


Figure 2: Surface expression of hACE2 by A549-hACE2 cells. $\sim 2 \times 10^5$ A549 (wild-type) and A549-hACE2 cells were incubated with 1 μ g of Spike-S1-Fc or CTLA-4-Fc fusion proteins for 1h at 4°C. Cells were then washed and incubated with 0.5 μ g of a goat anti-hlgG1-Fc antibody coupled to PE for 1h at 4°C. Cell surface staining was analyzed by flow-cytometry.

Infection of A549-hACE2 cells by SARS-CoV-2 Spike pseudotyped lentiviral particles

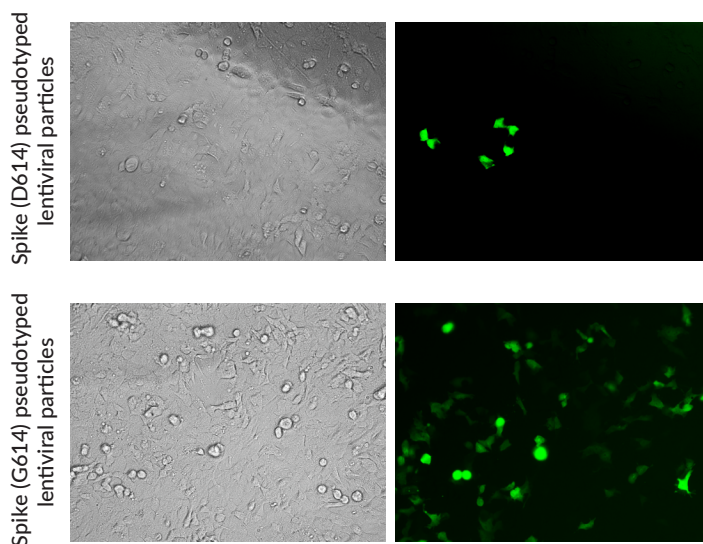


Figure 3: Infection of A549-hACE2 cells by Spike (D614 or G614) pseudotyped lentiviral particles. $\sim 2 \times 10^4$ A549-hACE2 cells were cultured in the presence of SARS-CoV-2 Spike D614-variant (up panels) or Spike G614-variant (bottom panels) pseudotyped GFP lentiviral particles. After 72h, the transduction efficiency of the Spike pseudotyped GFP particles was evaluated by fluorescence microscopy.

TECHNICAL SUPPORT

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