Validation data for RBD-LuciaV5 (P.1)

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Version 21I17-NJ

RBD-LuciaV5 (P.1) is a soluble fusion protein composed of the Spike Receptor Binding Domain (RBD) from the SARS-CoV-2 Gamma variant (P.1) fused to a C-terminal Lucia luciferase reporter.This protein has been specifically designed to assess the binding affinity of anti-Spike antibodies using either ELISA **(Fig. 1)** or the solution phase assay LIPS (luciferase immunoprecipitation systems).

RBD-LuciaV5 (P.1) for a Luciferase-based ELISA

Unlike a conventional ELISA, the plate was coated overnight with an Anti-murine IgG F(ab')2 fragment as the 'capture antibody'. InvivoGen's collection of clinically-relevant anti-spike monoclonal antibodies (mAbs) were added and the binding affinity of these mAbs to RBD-LuciaV5 (P.1) was assessed using the Lucia luciferase activity.

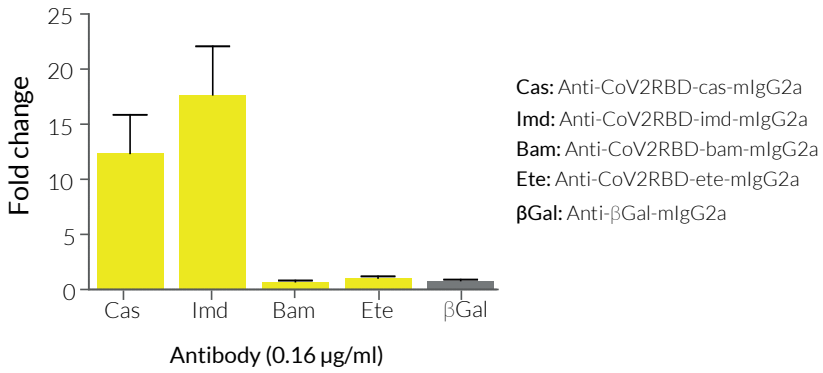

Figure 1: Luciferase-based ELISA using RBD-LuciaV5 (P.1). Anti-murine IgG F(ab')2 fragment (2 µg/ml) was coated on an ELISA plate overnight. Anti-CoV2RBD-cas-mIgG2a, Anti-CoV2RBD-imd-mIgG2a, Anti-CoV2RBD-bam-mIgG2a, Anti-CoV2RBD-ete-mIgG2a, or the negative control Anti-βGal-mIgG2a, along with RBD-LuciaV5 (P.1)(1 µg/ml) were added and incubated for 2 hours at room temperature. After washing (3x times), binding affinity was assessed by measuring the activity of Lucia luciferase in the supernatant using QUANTI-Luc™. Data are shown as a fold change over no antibody.

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