

Validation data for RBD-LuciaV7 (B.1.617.1)

<https://www.invivogen.com/b16171-rbd-lucia>

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Version 21117-NJ

RBD-LuciaV7 (B.1.617.1) is a soluble fusion protein composed of the Spike Receptor Binding Domain (RBD) from the SARS-CoV-2 Kappa variant (B.1.617.1) 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) (Fig. 2).

RBD-LuciaV7 (B.1.617.1) 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 (mAb) were added and the binding affinity of these mAbs to RBD-LuciaV7 (B.1.617.1) was assessed using the Lucia luciferase activity.

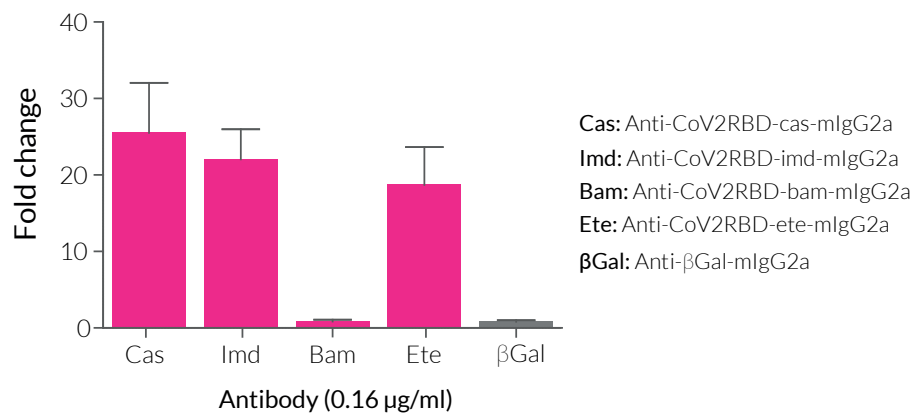


Figure 1: Luciferase-based ELISA using RBD-LuciaV7 (B.1.617.1). Anti-murine IgG F(ab')₂ fragment (2 µg/ml) was coated on an ELISA plate overnight. Anti-CoV2RBD-cas-mIgG2a, Anti-CoV2RBD-imd-mIgG2a, Anti-CoV2RBD-bam-mIgG2a, Anti-CoV2RBD-ete-mIgG2a, or the negative control Anti-βGal-mIgG2a, along with RBD-LuciaV7 (B.1.617.1) (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.

RBD-LuciaV7 (B.1.617.1) for LIPS

RBD-LuciaV7 (B.1.617.1) can be used to detect anti-spike polyclonal antibodies in the sera of recovered COVID-19 patients and/or a vaccinee. Antibody-protein complexes are purified and quantification is easily determined by assessing the Lucia luciferase activity.

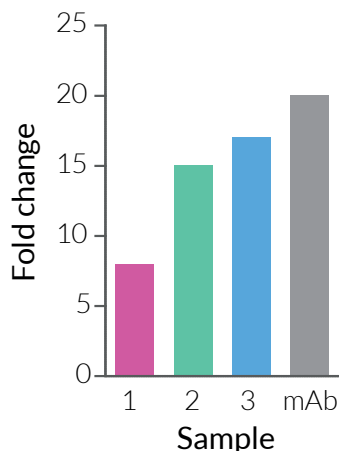


Figure 2: Detection of Spike antibodies in vaccinee sera by LIPS. RBD-LuciaV7 (B.1.617.1) (10 µg/ml) was mixed with either diluted serum from individuals vaccinated against SARS-CoV-2 (Sample 1-3) or Anti-CoV2RBD-imd-mIgG2a (mAb) diluted in 'negative' serum. Protein A beads were added to the mixture and incubated at room temperature for 2 hours with gnetly shaking. After extensive washing (6x times), detection of anti-Spike antibodies was assessed by measuring the activity of Lucia luciferase using QUANTI-Luc™. Data are shown as a fold change over 'negative' serum (or no antibody).

TECHNICAL SUPPORT

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