Validation data for RBD-LuciaV3 (B.1.351)

https://www.invivogen.com/b1351-rbd-lucia

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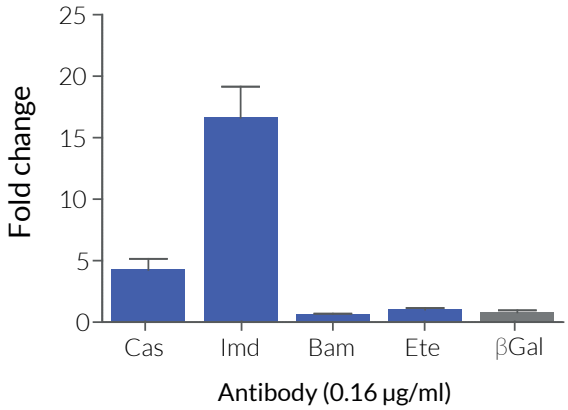
Version 21I17-NJ

RBD-LuciaV3 (B.1.351) is a soluble fusion protein composed of the Spike Receptor Binding Domain (RBD) from the SARS-CoV-2 Beta variant (B.1.351) 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-LuciaV3 (B.1.351) 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-LuciaV3 (B.1.351) was assessed using the Lucia luciferase activity. As expected, the Imdevimab-dervied mAb was the only mAb that displayed binding to RBD-LuciaV3 (B.1.351).



Cas: Anti-CoV2RBD-cas-mIgG2a
Imd: Anti-CoV2RBD-imd-mIgG2a
Bam: Anti-CoV2RBD-bam-mIgG2a
Ete: Anti-CoV2RBD-ete-mIgG2a
βGal: Anti-βGal-mIgG2a

Figure 1: Luciferase-based ELISA using RBD-LuciaV3 (B.1.351). 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-LuciaV3 (B.1.351) (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-LuciaV3 (B.1.351) for LIPS

RBD-LuciaV3 (B.1.351) 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.

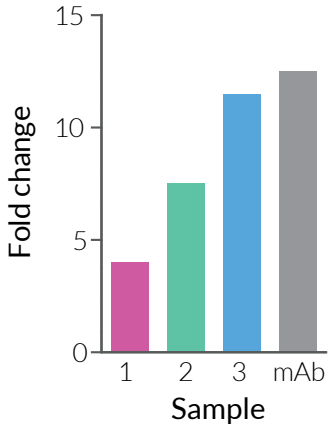


Figure 2: Detection of Spike antibodies in vaccinee sera by LIPS. RBD-LuciaV3 (B.1.351) (10 µg/ml) was mixed with either diluted serum from individuals vaccinated against SARS-CoV-2 (Sample 1-3) or Anti-CoV2RBD-imd-mIgG2a (mAb) diluted in 'negative' serum. Protein A beads were added to the mixture and incubated at room temperatur e 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
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3-622-34-80
E-mail: info@invivogen.com

InvivoGen
www.invivogen.com