Validation data for RBD-Lucia

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

For research use only

Version 21I17-NJ

RBD-Lucia is a soluble fusion protein composed of the Receptor Binding Domain (RBD) from the original SARS-CoV-2 Spike protein fused to a C-terminal Lucia luciferase reporter.This fusion 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-Lucia 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-Lucia was assessed using the Lucia luciferase activity.

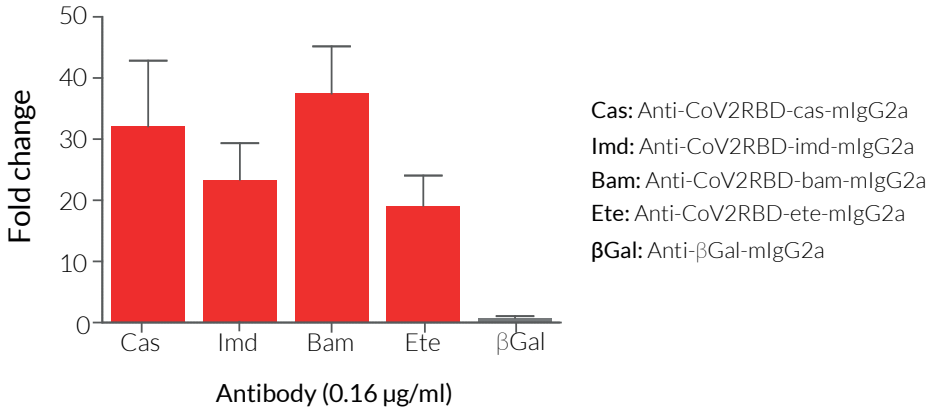

Figure 1: Luciferase-based ELISA using RBD-Lucia. Anti-murine IgG F(ab')2 fragment (2 µg/ml) was coated on an ELISA plate overnight. Anti-CoV2RBD-cas-mIgG2a, Anti-CoV2RBD-imd-mIgG2a, Anti-CoV2RBD-bam-mIgG2a, Anti-CoV2RBD-ete-mIgG2a, or the negative control Anti-βGal-mIgG2a, along with RBD-Lucia (1 µg/ml)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

RBD-Lucia 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.

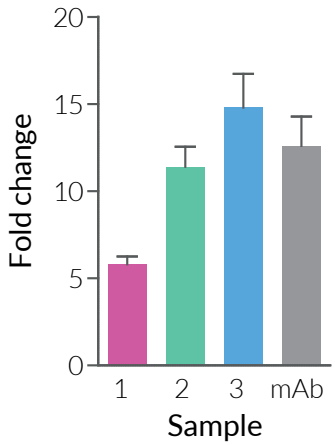

Figure 2: Detection of Spike antibodies in vaccinee sera by LIPS. RBD-Lucia (10 µg/ml) was mixed with either diluted serum from individuals vaccinated against SARS-CoV-2 (Sample 1-3) or Anti-CoV2RBD-imd-mIgG2a (mAb) diluted in 'negative' serum. Protein A beads were added to the mixture and incubated 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 in the supernatant using QUANTI-Luc™. Data are shown as a fold change over 'negative' serum (or no antibody).

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