

VALIDATION DATA



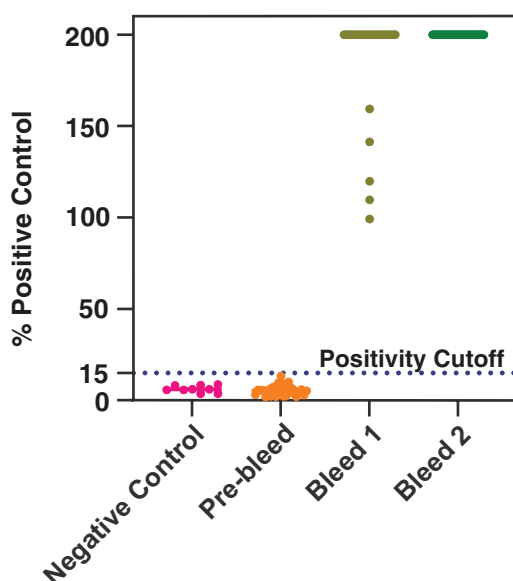
SARS-CoV-2 Spike RBD IgG (mouse) ELISA Kit

Item No. 502820

The SARS-CoV-2 Spike RBD IgG (mouse) ELISA Kit has been validated in serum from 32 mice immunized with two injections of Spike RBD glycoprotein administered three weeks apart. Serum was collected at baseline (pre-bleed), three weeks after the first injection (bleed 1), and three weeks after the second injection (bleed 2).

Qualitative measurements of mouse serum

Mouse sera were prepared as described in the kit booklet and absorbance values are shown relative to the provided positive control. Samples clustered at 200% reflect absorbance values that exceeded the range of the plate reader when diluted 1:100.



WARNING

THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

SAFETY DATA

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent via email to your institution.

WARRANTY AND LIMITATION OF REMEDY

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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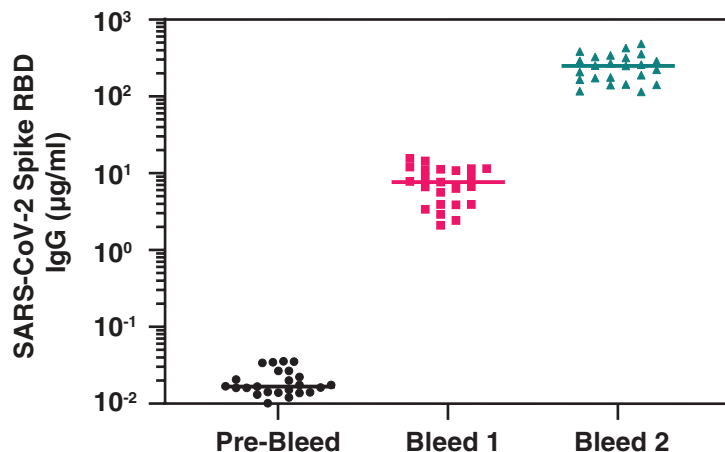
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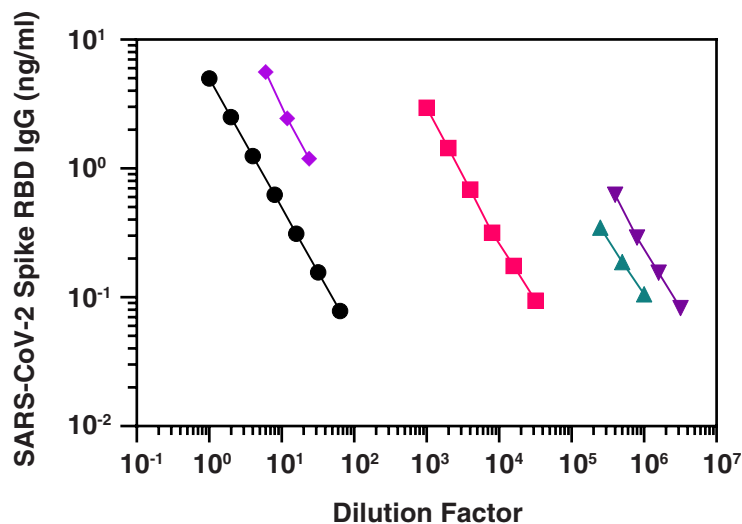
Semi-quantitative measurements of SARS-CoV-2 Spike RBD IgG in immunized mice

SARS-CoV-2 Spike RBD IgG levels were quantified in sera from 32 immunized mice.



Parallelism (Semi-Quantitative Format)

To assess parallelism, mouse sera were assayed at multiple dilutions using the SARS-CoV-2 Spike RBD IgG (mouse) ELISA Kit. Measured concentrations were plotted as a function of the sample dilution. The results are shown below. Results may vary due to differences in antibody affinity or specificity from the supplied standard.



- Standard
- Positive Mouse Serum (1)
- ▲ Positive Mouse Serum (2)
- ▼ Positive Mouse Serum (3)
- ◆ Negative Mouse Plasma

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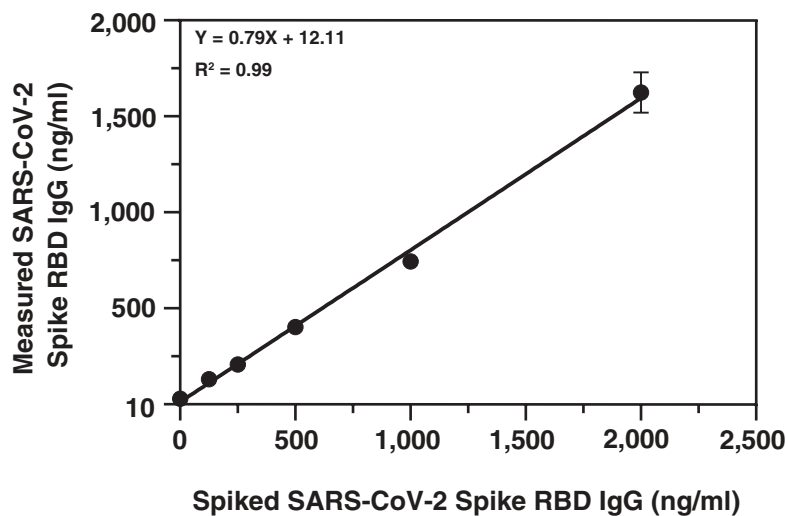
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Spike and Recovery (Semi-quantitative Format)

Mouse serum with low levels of Spike RBD IgG (bleed 1) were mixed with mouse serum with high levels of Spike RBD IgG (bleed 2) at the ratios listed below. Mixes were diluted with Assay Buffer and analyzed using the SARS-CoV-2 Spike RBD IgG (mouse) ELISA Kit. The results are shown in the table below. The expected concentrations were calculated from the indicated ratios.

Bleed 1 (%)	Bleed 2 (%)	Expected (ng/ml)	Measured (ng/ml)	% of Expected
100	0	--	2,800	--
80	20	21,000	18,700	88%
60	40	39,800	36,500	92%
40	60	58,300	56,900	98%
20	80	76,800	75,300	98%
0	100	--	95,300	--

Mouse plasma was spiked with Spike RBD IgG, serially diluted with Assay Buffer, and evaluated using the SARS-CoV-2 Spike RBD IgG (mouse) ELISA Kit. The error bars represent standard deviations obtained from multiple dilutions of each sample.



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Linearity (Semi-quantitative Assay)

Mouse sera positive for Spike RBD IgG were serially diluted with Assay Buffer and evaluated for linearity using the SARS-CoV-2 Spike RBD IgG (mouse) ELISA Kit.

Dilution Factor	Measured Concentration (ng/ml)	Linearity%
Serum three weeks post first immunization		
10,000	7,000	100
20,000	8,100	116
40,000	8,400	120
80,000	7,900	113
Serum three weeks post second immunization		
125,000	258,000	100
250,000	281,000	109
500,000	299,000	116
1,000,000	314,000	122

NOTE: Linearity has been calculated using the following formula:

$\% \text{Linearity} = (\text{Observed concentration value, dilution adjusted} / \text{First observed concentration value in the dilution series, dilution adjusted}) * 100$

Precision (Qualitative Assay)

Intra-assay precision was determined by analyzing 24 replicates of the positive and negative controls in a single assay.

Control	Absorbance (450 nm)	%CV
Positive	2.515	3.1
Negative	0.249	4.2

Inter-assay precision was determined by analyzing replicates of the positive and negative controls in eight separate assays on different days.

Control	Absorbance (450 nm)	%CV
Positive	2.355	8.8
Negative	0.249	3.0

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Precision (Semi-Quantitative Assay)

Intra-assay precision was determined by analyzing 24 replicates of three matrix controls (mouse serum) in a single assay.

Control	Measured SARS-CoV-2 Spike RBD IgG (ng/ml)	%CV
Control 1	13,241	3.6
Control 2	1,230	5.2
Control 3	218	5.7

Inter-assay precision was determined by analyzing replicates of three matrix controls (mouse serum) in eight separate assays on different days.

Control	Measured SARS-CoV-2 Spike RBD IgG (ng/ml)	%CV
Control 1	18,700	7.2
Control 2	1,700	13.6
Control 3	260	10.3

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