Bio-Plex[™] Pro Human IgG SARS-CoV-2 Serology Assays Protocol

Introduction

This quantitative protocol is for use with Bio-Plex Pro Human IgG SARS-CoV-2 N/RBD/S1/S2 Serology Assays. Recommendations are included about how to use the VIROTROL SARS-CoV-2 serological control as a reference standard to generate U/ml quantitation values.

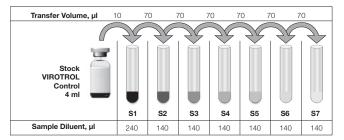
Required Materials

- VIROTROL SARS-CoV-2 Single Level Control (Bio-Rad[™] Laboratories, Inc., catalog #200300A)
- Bio-Plex Pro Human IgG SARS-CoV-2 N/RBD/S1/S2 4-Plex Panel (Bio-Rad, #12014634)

Standard Curve Preparation

Run a seven-point standard curve starting at a 1:25 dilution of the stock VIROTROL. The control comes in liquid form at 4 ml, so one vial of VIROTROL should be enough for approximately 400 assays when S1 is run in duplicate or triplicate.

- Dilute the contents of the vial fivefold up to a minimum volume of 250 µl. To make S1, add 10 µl of stock VIROTROL Control to 240 µl serology sample diluent.
- 2. Make threefold serial dilutions from S1 up to seven standard curve points. The volumes provided are for duplicate standard curve points.
 - a. Fill tubes for S2–S7 with 140 µl serology sample diluent.
 - b. To make S2, add 70 µl of S1 to 140 µl serology sample diluent.
 - c. Repeat the serial dilution until you reach S7 by adding 70 μl of the prior standard curve point dilution to 140 μl of the following standard curve point.



VIROTROL Control standard curve dilution scheme.

Standard Curve Point	Threefold Serial Dilution	Value Assignment (U/ml)
S1	1:25	4,000.0
S2	1:75	1,333.3
S3	1:225	444.4
S4	1:675	148.1
S5	1:2,025	49.4
S6	1:6,075	16.5
S7	1:18,225	5.5

Sample Preparation

Adjust the sample dilution scheme as needed for very high- and low-level anti–SARS-CoV-2 N/RBD/S1/S2 IgG samples. Dilutions of 1:100 to 1:1,000 have worked, depending on the antibody concentration in samples.

For example, for a 1:1,000 dilution:

- 1. Dilute 100-fold: Add 2 µl of stock to 198 µl serology sample diluent.
- 2. Dilute 10-fold: Add 20 μl of 1:100 dilution to 180 μl serology sample diluent.

For bead, detection antibody, and streptavidin-phycoerythrin preparation, refer to the Bio-Plex Pro Human SARS-CoV-2 Serology Assays Quick Guide (10000133854) or Instruction Manual (10000133853).

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Visit bio-rad.com/SARSCoV2Serology for more information.

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