# Bio-Plex Pro Human IgA and IgM SARS-CoV-2 Serology Assays

Protocol

## Introduction

This quantitative protocol is for use with Bio-Plex Pro Human IgA and IgM SARS-CoV-2 N/RBD/S1/S2 Serology Assays in measuring antibodies specific to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Researchers can use Bio-Plex Pro Human IgA and IgM SARS-CoV-2 Positive Controls as reference standards to generate ng/ml quantitation values.

## Required Materials

- Bio-Plex Pro Human IgA SARS-CoV-2 Positive Control (Bio-Rad Laboratories, Inc., catalog #12014775)
- Bio-Plex Pro Human IgM SARS-CoV-2 Positive Control (Bio-Rad, #12014776)
- Serology sample diluent (included in each kit)

## Standard Curve Preparation

The positive control comes in ready-to-use liquid form at 250 µl and serves as the stock control.

### IgA Standard Curve Preparation

1. Use the ready-to-use IgA positive control as S1.
2. Make threefold serial dilutions from S1 up to seven standard curve points (Figure 1). See Table 1 for expected concentrations.
   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 dilution to 140 µl of the next standard curve point.

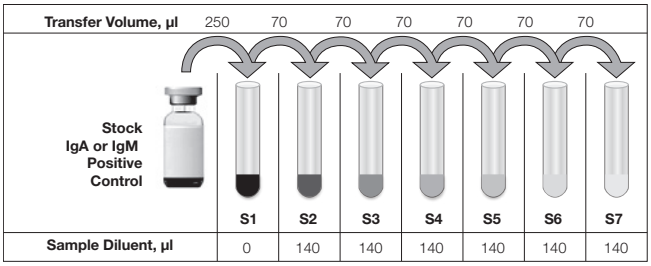


**Fig. 1. IgA or IgM Positive Control standard curve dilution scheme.**

**Table 1. Threefold serial dilutions for IgA Positive Control S1–S7 standard curve points.**

| | N (20)* | RBD (36)* | S1 (28)* | S2 (42)* |
|---|---|---|---|---|
| Standard Curve Point | Expected Concentration, ng/ml | | | |
| S1 | 400 | 300 | 300 | 800 |
| S2 | 133.33 | 100 | 100 | 266.67 |
| S3 | 44.44 | 33.33 | 33.33 | 88.89 |
| S4 | 14.81 | 11.11 | 11.11 | 29.63 |
| S5 | 4.94 | 3.7 | 3.7 | 9.88 |
| S6 | 1.65 | 1.23 | 1.23 | 3.29 |
| S7 | 0.55 | 0.41 | 0.41 | 1.1 |

* Bead regions.
N, nucleocapsid; RBD, receptor binding domain; S1, spike 1; S2, spike 2.

### IgM Standard Curve Preparation

1. Use the ready-to-use IgM positive control as S1.
2. Make threefold serial dilutions from S1 up to seven standard curve points (Figure 1). See Table 2 for expected concentrations.
   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 dilution to 140 µl of the next standard curve point.

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Not for use in diagnostic procedures.



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**Table 2. Threefold serial dilutions for IgM Positive Control S1–S7 standard curve points.**

| | N (20)* | RBD (36)* | S1 (28)* | S2 (42)* |
|---|---|---|---|---|
| Standard Curve Point | Expected Concentration, ng/ml | | | |
| S1 | 1,000 | 1,000 | 1,000 | 1,000 |
| S2 | 333.33 | 333.33 | 333.33 | 333.33 |
| S3 | 111.11 | 111.11 | 111.11 | 111.11 |
| S4 | 37.04 | 37.04 | 37.04 | 37.04 |
| S5 | 12.35 | 12.35 | 12.35 | 12.35 |
| S6 | 4.12 | 4.12 | 4.12 | 4.12 |
| S7 | 1.37 | 1.37 | 1.37 | 1.37 |

* Bead regions.
N, nucleocapsid; RBD, receptor binding domain; S1, spike 1; S2, spike 2.

## Sample Preparation

Dilute samples 1:100 by adding 2 µl of sample to 198 µl serology sample diluent.

**Note:** Adjust the sample dilution scheme as needed for very high- and low-level anti–SARS-CoV-2 N/RBD/S1/S2 IgA and IgM samples.

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

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



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