COVID-19 Spike-ACE2 Binding Assay Kit Controls

Positive and negative controls for the SARS-CoV-2 Spike-ACE2 inhibitor screening ELISA kits

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I. INTRODUCTION

The **COVID-19 Spike-ACE2 Binding Assay Kit Controls** can be used with *all* RayBio® Spike-ACE2 Binding Assay Kits (cat no. CoV-SACE2, CoV-ACE2S2, CoV-SM1ACE2, CoV-SM2ACE2, CoV-SM3ACE2, CoV-SM4ACE2, CoV-SM5ACE2, CoV-ACE2SM6). The positive control will inhibit the Spike-ACE2 interaction whereas the negative control will *not* inhibit the Spike-ACE2 interaction. Both controls are purified antibodies.

This manual is an addendum to the Spike-ACE2 Binding Assay Kit manuals and is intended to be used in conjunction with the Spike-ACE2 Binding Assay Kit manuals.

Note: The Spike-ACE2 interaction is inhibited more by the positive control in the presence of wild-type Spike than the Spike variants.

II. STORAGE

The controls may be stored at 4°C up to 1 month from the date of shipment. For long-term storage up to 1 year from the date of shipment, store the controls at -20°C.

III. MATERIALS PROVIDED

COMPONENT	OIZE / DEGORII TION	STORAGE/ STABILITY AFTER PREPARATION
Positive Control (Item R)	1 vial of liquid solution, 10 μL at 0.1 mg/ml	3 days at 4°C
Negative Control (Item S)	1 vial of liquid solution, 10 μL at 0.1 mg/ml	3 days at 4°C

IV. ADDITIONAL MATERIALS REQUIRED

Refer to the manual of the Spike-ACE2 Binding Assay Kit that you will be using (cat no. CoV-SACE2, CoV-ACE2S2, CoV-SM1ACE2, CoV-SM2ACE2, CoV-SM3ACE2, CoV-SM4ACE2, CoV-SM5ACE2, CoV-ACE2SM6).

V. TEST REAGENT SAMPLE PREPARATION

1. Refer to "Test Reagent Sample Preparation" (Section VI) of the corresponding Spike-ACE2 Binding Assay Kit manual.

- 2. Dilute the Positive Control (Item R) and Negative Control (Item S) 100-fold to a final concentration of 1 μg/ml following these steps:
 - a. Prepare 1x Protein Working Solution of Item F (i.e., the Spike or ACE2 protein that will be added in solution to the plate). For details on how to prepare the 1x Protein Working Solution, see Section V, "Reagent Preparation" in the corresponding Spike-ACE2 Binding Assay Kit.

Note: Item F and Assay Diluent required for this step are provided in the Spike-ACE2 Binding Assay Kit.

- b. **Aliquot the 1x Protein Working Solution**: Place 99 µL of 1x Protein Working Solution into *two* properly-labeled microcentrifuge tubes.
- c. **Spin down the controls briefly** (i.e., 1-3 seconds) to collect the contents to the bottom of the vials.
- d. **Dilute Positive Control:** Add 1 μ L of the Positive Control (Item R) to one tube containing 99 μ L of 1x Protein Working Solution. Close cap, invert 5 6 times to mix.
- e. **Dilute Negative Control:** Add 1 μ L of the Negative Control (Item S) to one tube containing 99 μ L of 1x Assay Diluent. Close cap, invert 5 6 times to mix.
- 3. Add **100 µl** of the Positive Control (Item R) and Negative Control (Item S) into an appropriate well on the 96-well plate.

Note: For data reliability, it is recommended that all Test Reagent samples should be run in at least duplicate.

4. The kit should then be processed according to Section VII, "Assay Procedure," in the corresponding Spike-ACE2 Binding Assay Kit.

VI. DATA ANALYSIS

Refer to the manual of the Spike-ACE2 Binding Assay Kit that you will be using (cat no. CoV-SACE2, CoV-ACE2S2, CoV-SM1ACE2, CoV-SM2ACE2, CoV-SM3ACE2, CoV-SM4ACE2, CoV-SM5ACE2, CoV-ACE2SM6).

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