Human ACE2 ELISA (Catalog Number EH489RB (96 tests)

Materials

- Human ACE2 Antibody Coated wells, 96-well plate
- Human ACE2 Biotin Conjugate
- Human ACE2 Standard, recombinant human ACE2
- Assay Diluent (5X)
- Streptavidin-HRP (150X)
- TMB Substrate
- Stop Solution
- Wash Buffer Concentrate (20X)
- Adhesive Plate Covers
- Plate washer-automated or manual (manifold dispenser)
- Microtiter plater reader with software capable of measuring at 450 nm
- Deionized water

Sample Preparation Guidelines:

- If samples will not be tested immediately, freeze them after collection.
- Avoid multiple freeze–thaw cycles of frozen samples.
- Thaw samples completely and mix gently (do not vortex) prior to analysis.
- Do not use hemolyzed or lipemic sera.
- Pre-dilute samples using 1X Assay Diluent for serum, plasma, and cell culture supernatant samples.
- Dilute serum 8-fold.

Prepare Biotin Conjugate:

1. Briefly spin down the biotin conjugate before use.
2. Add 100 µL of 1× Assay Diluent to the vial to prepare a biotin conjugate concentrate.
3. Gently pipette up and down to mix. (The concentrate can be stored at 4 °C for up to 5 days.)
4. Dilute the biotin conjugate concentrate 80-fold with 1× Assay Diluent and use it in Step 2 of the ELISA procedure.

Dilute Standard

1. Briefly spin down the vial of lyophilized standard.
2. Add 600 µL of 1X Assay Diluent (the Assay Diluent should be diluted 5-fold with deionized or distilled water before use) to the vial to prepare a 20 ng/mL standard solution. Dissolve the powder thoroughly by gently mixing.
3. Pipette 400 µL of 1X Assay Diluent into each dilution tube.
4. Use the 20 ng/mL standard solution to prepare a dilution series (as shown below), mixing each tube thoroughly before transferring to the next.
5. Use 1X Assay Diluent as the zero standard (0 pg/mL).

Prepare 1× Streptavidin-HRP Solution

1. Briefly spin the Streptavidin-HRP vial and gently pipette up and down to mix before use, as precipitates may form during storage.
2. Dilute the Streptavidin-HRP 150-fold with 1× Assay Diluent.
3. Do not store the diluted solution for future use; prepare fresh each time.

Methods

1. For the standard curve, add 100 µL of each standard to the appropriate wells For samples, add 100 µL of diluted samples to the designated wells.

2. Cover the plate and incubate for 2.5 hours at room temperature or overnight at 4 °C with gentle shaking.

3. Discard the solution and wash the plate four times with 1× wash Buffer. Fill each well with 300 µL of wash Buffer using a multi-channel pipette or an auto washer. Complete removal of liquid at each wash is critical for optimal performance. After the final wash, remove any residual buffer by aspiration or decanting. Invert the plate and gently blot against clean paper towels.

4. Add 100 µL of prepared biotin conjugate to each well.

5. Incubate for 1 hour at room temperature with gentle shaking.

6. Discard the solution and repeat the wash

7. Add 100 µL of prepared Streptavidin-HRP solution to each well.

8. Incubate for 45 minutes at room temperature with gentle shaking.

9. Discard the solution and repeat the wash

10. Add 100 µL of TMB Substrate to each well. The solution will start to turn blue.

11. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

12. Add 50 µL of Stop Solution to each well and gently tap the plate to mix. The color will change from blue to yellow.

13. Read the absorbance at 450 nm. Read the plate within 30 minutes after adding the Stop Solution.