

ELISA Pair Testing Protocol

(Service provided by Shikhar Biotech)

1 MATERIALS

- 1.1 dH₂O from USF Elga, Model MAXIMA LS
- 1.2 Antigens/Antibodies
- 1.3 TMB substrate (Thermofisher)
- 1.4 0.1M Tween-20 (Sigma, Cat # P7949)
- 1.5 PBS (1.9mM NaH₂PO₄, 8.1mM Na₂HPO₄, 0.154M NaCl), pH7.3
- 1.6 Blocking Buffer (PBS with 3% (w/v) BSA)
- 1.7 70% Industrial Methylated Spirit (IMS) (Pharmaco Industries or similar)
- 1.8 15 ml Centrifuge Tubes (Greiner or similar)
- 1.9 1.5 ml Micro centrifuge Tubes (Greiner Cat No 616021 or similar)
- 1.10 10ul Pipette Tips (Greiner or similar)
- 1.11 1000ul Pipette Tips (Greiner or similar)
- 1.12 200ul Pipette Tips (Greiner or similar)
- 1.13 Disposable Gloves.
- 1.14 Aluminium foil
- 1.15 HRP-conjugated secondary antibody

2 EQUIPMENT

- 2.1 ELISA plate washer (Washwell-Robonik)
- 2.2 Fine balance (Scaltec Model SBC 31 or similar)
- 2.3 pH meter (Orion Model 320 or similar)
- 2.4 Plate reader (Readwell –Robonik)
- 2.5 Spray Gun (Kautex or similar)
- 2.6 Permanent Marker
- 2.7 Adjustable Pipette 1-10ul (Biohit, Proline)
- 2.8 Adjustable Pipette 100-1000ul (Biohit, Proline)
- 2.9 Adjustable Pipette 20-200ul (Biohit, Proline)
- 2.10 37°C Incubator (Mettler or similar)
- 2.11 Wash Bottles
- 2.12 Multi-channel pipette
- 2.13 +4°C storage facilities
- 2.14 Tip Boxes
- 2.15 Plate shaker

3 ENVIRONMENT

- 3.1 ISO 9001:2015 Compliant Laboratory, Shikhar Biotech Pvt Ltd, Kathmandu, Nepal

4 PROCEDURE

- 4.1 Prepare a 10 µg/ml EB13088 antibody in the PBS, pH 7.3
- 4.2 Using a multichannel pipette, add 100µl of the 10µg/ml EB13088 solution in each well of 96 well plate. Cover the wells and incubate overnight at 4°C.

- 4.3 Aspirate the solution and wash the plate twice with 200 μ l of PBS in each well using a multichannel pipette.
- 4.4 Fill the wells with 200 μ l Blocking Buffer (3% BSA in PBS) and incubate for 2 hrs at 37°C.
- 4.5 Remove the blocking solution by multichannel pipette and wash twice with PBST.
- 4.6 Prepare 8 serially diluted recombinant S1 proteins (1 μ g/ml, 0.5 μ g/ml, 0.25 μ g/ml, 0.125 μ g/ml, 0.0625 μ g/ml, 0.0312 μ g/ml, 0.015 μ g/ml and 0 μ g/ml).
- 4.7 Add 100 μ l of serially diluted SARS-CoV2 S1 protein in the respective wells. Then, cover the wells and incubate at 37°C for 2 hours.
- 4.8 Wash the wells 3 times with 200 μ l of PBST.
- 4.9 Add 100 μ l of the 1:2000 fold diluted HRP-conjugated Ab01680 antibody in each well. Cover the wells with aluminium foil and incubate at 37°C for 1 hour.
- 4.10 Aspirate the wells and wash the wells three times with PBST (200 μ l) and two times with PBS.
- 4.11 Add 100 μ l of TMB substrate per well and incubate at room temperature for 15-20 min (don't exceed more than 30 min).
- 4.12 Add 100 μ l of 1 N HCl as stop solution.
- 4.13 Read the OD in the plate reader at 450 nm immediately (within 5 minutes).

Note: The user can use the starting concentration of recombinant S1 protein as 0.5 μ g/ml or 1 μ g/ml or 2 μ g/ml and do serial dilution as per the requirement.