ELISA pair testing protocol

(Service provided by Shikhar Biotech)

1 MATERIALS

1.1 dH_2O 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 Microcentrifuge 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

1.16 Glycerol

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 (Memmert 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 100 μl of the 10 μg/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 200µl of PBS in each well using a multichannel pipette.

4.4 Fill the wells with 200µl 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 (2ug/ml, 1µg/ml, 0.5µ/ml, 0.25µg/ml, 0.125µg/ml, 0.0625µg/ml, 0.0312µg/ml, 0.015µg/ml and 0µg/ml)

4.7 Add 100 µl 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 200µl of PBST.

4.9 Add 100µl of the 1:1000 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 (200ul) and two times with PBS.

4.11 Add 100 µl of TMB substrate per well and incubate at RT for 15-20 min (don't exceed more than 30 min).

4.12 Add 100 µl 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 0.5µg/ml or 1µg/ml or 2µg/ml and do serial dilution as per the requirement.