Indirect Standard ELISA Protocol

I. Apply Antigen

1. Prepare an antigen solution in 0.05M Sodium carbonate buffer, pH 9.6* (1-10 µl /ml depending on antigen nature). Calculate total volume sufficient for coating all assay plates. Coat each well with 100µl of antigen solution. Cover the plate with adhesive film and incubate either at +4°C overnight or at RT for 2 hours (alternatively at 37°C for 1 hour) with shaking plates on a rocking platform at least for 30 min at moderate speed.

2. Wash the plates 3 times with PBS-T* using an automatic washer, or empty plates by shaking coating solution out of wells and filling wells with 200µl of PBS-T three times. As a final step, tap plates on paper towels to remove excess buffer.

II. Block plate

1. Block wells by adding 200µl of blocking solution (0.25% Gelatin in PBS-T), seal and incubate for 1 hour at RT on rocking platform.

2. Wash the plates 3 times with PBS-T, as in Section I, step 2, of this protocol.

III. React Primary Antibody

1. Add 100µl diluted with blocking solution primary antibody (hybridoma culture supernatant can be diluted 1:3) to each well, seal plates and incubate for 1 hour at RT at rocking platform.

2. Wash plates 3 times with PBS-T, as in Section I, step 2, of this protocol.

IV. Apply Secondary Antibody

1. Dilute secondary anti-mouse antibody with blocking solution at dilution as recommended by manufacturer. Add 100µl per each well, seal and incubate for 1 hour at RT on rocking platform.

2. Wash plates 3 times with PBS-T, as in Section I, step 2, of this protocol.

V. Add chromogenic substrate and develop

1. Prepare TMB* solution in 0.1M Sodium acetate buffer, pH 5.2* and filter through 0.45µm filter. Add 30% hydrogen peroxide to final concentration of 0.01% acetate. Immediately add 100µl per each well and allow to develop at RT for 5, 15, 30 min.

2. If desired, after color development add 50µl of stop solution, 10% (v/v) phosphoric acid.

3. Read plates with a plate reader.

*SOLUTIONS:

1) Coating Buffer, 0.05M Sodium carbonate buffer, pH 9.6 1.59g Na_2CO_3 + 2.93g $NaHCO_3$ + 0.1g NaN_3 Adjust to 1L by dH2O. Keep at +4°C	2) PBS-T, Phosphate Buffered Saline, pH 7.4, containing 0.05% Tween-20
3) 3M Sodium Acetate, pH 5.2 408.3g of Sodium Acetate· 3H2O dissolve in 800ml dH2O, adjust pH to 5.2 with glacial acetic acid. Adjust volume to 1L, autoclave.	4) TMB – 3',3,'5,5'-tetramethylbenzidine Stock solution -100mg TMB dissolve in 10ml of DMSO. Aliquot and store in dark vials at +4°C For developing mix *ex tempore* 100µl of TMB and 9.9 ml of 0.1M Sodium acetate