

**ELISA PROTOCOL
FOR "AB-T" ANTIBODIES**

1. Coat maxisorp well plates (Nunc) with a solution of antigen (the dilutions for antigen range from 4-50 μ g/ml, please see data sheet) in sodium carbonate buffer 0.05 M (pH 9.6) containing sodium metabisulfite (SMB) (Acros) 0.001 M, for sixteen hours at 4°C.
2. Saturate well plates with a solution of PBS (pH 7.3) containing 2.5 g/l of BSA (Acros), 0.05% Tween 20 (Acros) and SMB 0.001 M for one hour at 37°C.
3. Wash with PBS/0.5% Tween three times.
4. Dilute primary antibody as per antibody data sheet in PBS containing 2.5 g/l BSA, 10% of glycerol and SMB 0.001 M, 200 μ l by well plate (incubating for 2 hours at 37°C).
5. Wash with PBS/0.5% Tween three times.
6. 200 μ l of peroxidase-labeled goat anti- "primary antibody host species" IgG (Sigma) diluted (1/10,000) in a solution of PBS containing 2.5 g/l BSA, 10% of glycerol, 0.5% Tween and SMB 0.001 M, will be applied by well plate (for one hour at 37°C).
7. Rinse well plates with PBS/0.5% Tween three times.
8. And finally, develop the peroxidase by incubating 200 μ l by well plate of a citrate 0.1 M/phosphate, 0.2 M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark. After that, stop the reaction by the addition of 50 μ l of 2 M HCl.
9. The optical density will be measured at 492 nm, to obtain the different values (IC50).