Enzyme Linked Immunosorbent Assay (ELISA)

I. Preparation of Solutions and Reagents

1. **Coating Buffer:** Carbonate buffer (pH 9.6)
2. **Wash Buffer:** PBST buffer. Mix PBS buffer with Tween-20 at a ratio of 500 μL Tween-20 per 1 L PBS. Adjust pH to 7.2–7.4, and filter sterilize using a 0.22 μm membrane.
3. **Dilution Buffer:** 0.1% BSA solution, prepared by dissolving BSA in PBST buffer.
4. **Blocking Buffer:** 1% BSA solution, prepared by dissolving BSA in PBST buffer.
5. **Substrate Storage Solution:** 10 mg/mL TMB, prepared by dissolving TMB in DMSO.
6. **Substrate Dilution Buffer:** Prepared from 0.1 M Na_2HPO_4 and 0.1 M citric acid, pH 5.0.
7. **Stop Solution:** 2N H_2SO_4

II. Experimental Procedure

1. **Coating**: Dilute the specific capture antibody to an appropriate concentration using coating buffer (refer to the product website for recommended dilution). Add 100 μL of diluted antibody to each well of the ELISA plate. Incubate overnight at 4°C or for 2 hours at 37°C.
2. **Washing:** Discard the liquid in the wells. Add 300 μL of wash buffer to each well, gently shake for 2 minutes, then discard. Repeat this step 3–5 times.
3. **Blocking:** Add 200 μL of blocking buffer to each well. Incubate at 37°C for 1–2 hours.
4. **Washing:** Repeat the washing step as described above (300 μL/well, 2 min shaking, 3–5 times).
5. **Adding Sample:** Dilute the samples and standards to appropriate concentrations using sample diluent. Add 100 μL of the diluted solution to each well. Include a blank control well (add 100 μL of sample diluent only). Incubate at 37°C for 1–2 hours.
6. **Washing:** Repeat the washing step as described above.
7. **Adding Detection Antibody:** Dilute the HRP-conjugated detection antibody to the appropriate concentration using dilution buffer. Add 100 μL to each well. Incubate at 37°C for 1–2 hours.
8. **Washing:** Repeat the washing step as described above.
9. **Colorimetric reaction:** Add 100 μL of substrate solution to each well. Incubate at 37°C in the dark for 10–20 minutes.
10. **Stop Reaction:** Add 50 μL of stop solution to each well to terminate the enzymatic reaction.
11. **Result Reading:** Measure the absorbance at 450 nm using a microplate reader. Plot the standard curve based on the absorbance values of the standards, and calculate the concentration of the target antigen in the samples accordingly.

III. Recommended Experimental Products

1. Websitefor Antibody Products: https://www.targetmol.com/all-antibodies
2. Dimethyl Sulfoxide (DMSO), T0341
3. Tween 20, T9526
4. TMB, T19071; TMB Dihydrochloride, T19069

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