Capture ELISA Protocol

Step-by-step procedure:
1. Apply capture antibody by adding antigen-specific antibody to appropriate wells (1µg/well). The antibody concentration should be 10µg/mL in coating buffer, the volume should be100µL/well.
2. Incubate the plate at 4°C overnight.
3. Add 250µL of blocking solution each well.
4. Incubate the plate at room temperature for 1 hour.
5. Empty the plate and wash the plate with PBST (0.05% Tween-20) once.
6. Dilute the analytes (recombinant protein) to 100ng, 30ng, 10ng, 3ng, 1ng, 0.3ng, 0.1ng, and 0.03ng/mL in diluents.
7. Add analytes to appropriate wells.
8. Incubate the plate at room temperature for 2 hours.
9. Empty and then wash the plate three times with PBST (0.05% Tween-20).
10. Apply detection antibody by adding tag-specific rabbit anti-GST antibodies to appropriate wells (1µg/mL, 100µL/well).
11. Incubate the microtiter plate at room temperature for 2 hours.
12. Empty and then wash the plate three times with PBST (0.05% Tween-20).
13. Apply secondary antibody by adding HRP conjugated goat anti-rabbit IgG (H+L) to appropriate wells.
14. Incubate the microtiter plate at room temperature for 1 hour.
15. Wash the plate 5 times with PBST (0.05% Tween-20).
16. Apply the substrate by adding 150 µL of substrate (OPD, 400µg/mL, 0.03% H2O2, citrate buffer)
17. Incubate at room temperature for 30 minutes.
18. Read absorbance at 450nm.

Coating Buffer:
PBS (137mM NaCl, 2.7mM KCl, 8.1mM Na2HPO4, 1.5mM KH2PO4)
Blocking Solution:
5% Skim Milk in PBST (0.05% Tween-20)
Diluent:
2% Skim MILK in PBST (0.02% Tween-20)
Citrate Buffer:
3.65g citric acid, 4.76g Na2HPO4 in 500ml d2H2O

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