

Product Information

Capture ELISA Procedure

Preparation Instructions

Coating Buffer – PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, and 1.5 mM KH₂PO₄)

PBST – PBS with 0.05% TWEEN® 20

Blocking Solution – 5% Skim Milk in PBS with 0.05% TWEEN 20

Diluent – 2% Skim Milk in PBS with 0.02% TWEEN 20

Citrate Buffer – 3.65 g of citric acid and 4.76 g of Na₂HPO₄ in 500 ml of water

Procedure

1. Apply capture antibody by adding antigen-specific antibody to appropriate wells (1 µg/well). The antibody concentration should be 10 µg/ml in Coating Buffer, the volume should be 100 µl/well.
2. Incubate the plate overnight at 2–8 °C.
3. Add 250 µl of Blocking Solution to each well.
4. Incubate the plate at room temperature for 1 hour.
5. Empty the plate and wash the plate with PBST once.
6. Dilute each analyte (recombinant protein) to 100 ng, 30 ng, 10 ng, 3 ng, 1 ng, 0.3 ng, 0.1 ng, and 0.03 ng/ml in diluent.
7. Add the diluted target 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.
10. Apply detection antibody by adding tag-specific rabbit anti-GST antibodies to appropriate wells (1 µg/ml, 100 µl/well).
11. Incubate the multiwell plate at room temperature for 2 hours.
12. Empty and then wash the plate three times with PBST.
13. Apply secondary antibody by adding HRP conjugated goat anti-rabbit IgG to appropriate wells.
14. Incubate the multiwell plate at room temperature for 1 hour.
15. Wash the plate 5 times with PBST.
16. Apply the substrate by adding 150 µl of substrate [Citrate Buffer with 400 µg/ml of OPD and 0.03% (v/v) H₂O₂].
17. Incubate at room temperature for 30 minutes.
18. Read absorbance at 450 nm.

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