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Product Information

Capture ELISA Procedure

Preparation Instructions

Coating Buffer – PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, and 1.5 mM KH_2PO_4)

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 Na_2HPO_4 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 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 IgG antibody 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) H_2O_2].
17. Incubate at room temperature for 30 minutes.
18. Read absorbance at 450 nm.

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