

Capture ELISA Protocols

SECTION 1 – Reagents

1.1 Coating Buffer

PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, 1.5mM KH₂PO₄)

1.2 Blocking Solution

5% Skim Milk in PBST (0.05% Tween-20)

1.3 Diluent

2% Skim MILK in PBST (0.02% Tween-20)

1.4 Citrate Buffer

3.65 g citric acid, 4.76 g Na₂HPO₄ in 500ml ddH₂O

1.5 Rabbit anti-GST antibody

Abnova's rabbit anti-GST antibody (Catalog # PAB1625-E01P)

1.6 HRP conjugated goat anti-rabbit IgG (H+L)

PIERCE Goat Anti-Rabbit IgG (H+L), Peroxidase Conjugated (Catalog # 31460)

SECTION 2 - Assay Protocol

Note: The following protocol is a guideline, user need to determine their optimal experimental condition for best performance.

2.1 Apply capture antibody by adding antigen-specific antibody to appropriate wells (1µg/well).The antibody concentration should be 10 µg/mL in coating buffer, the volume should be 100 µL/well.

※The capture antibody should be purified antibody.

2.2 Incubate the plate at 4°C overnight.

2.3 Add 250 µL of blocking solution each well.

2.4 Incubate the plate at room temperature for 1 hour.

2.5 Empty the plate and wash the plate with PBST (0.05% Tween-20) once.

2.6 If the analytes is:

- a、 Recombinant protein - dilute it to 100 ng, 30 ng, 10 ng, 3 ng, 1 ng, 0.3 ng, 0.1 ng, and 0.03 ng/mL in diluents.
- b、 Transfected lysate - dilute it at serial dilution folds to determine adequate dilution folds which fit the standard curve. Abnova usually uses 3X, 10X, 30X, 100X, 300X, 1000X, 3000X at this step.

2.7 Add analytes to appropriate wells.

2.8 Incubate the plate at room temperature for 2 hours.

2.9 Empty and then wash the plate three times with PBST (0.05% Tween-20).

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- 2.10 Apply detection antibody by adding tag-specific rabbit anti-GST antibodies to appropriate wells (2.5 µg/mL, 100 µL/well).
 - 2.11 Incubate the microtiter plate at room temperature for 2 hours.
 - 2.12 Empty and then wash the plate three times with PBST (0.05% Tween-20).
 - 2.13 Apply secondary antibody by adding HRP conjugated goat anti-rabbit IgG (H+L) to appropriate wells.
 - 2.14 Incubate the microtiter plate at room temperature for 1 hour.
 - 2.15 Wash the plate 5 times with PBST (0.05% Tween-20).
 - 2.16 Apply the substrate by adding 150 µL of substrate (OPD, 400 µg/mL, 0.03% H₂O₂, citrate buffer)
 - 2.17 Incubate at room temperature for 30 minutes.
 - 2.18 Read absorbance at 450 nm.