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ProductInformation

ANTI-PROTEIN 4-
PEROXIDASE CONJUGATE
Antibody developed in goat
IgG fraction of antiserum

Product Number **P 5867**
Storage Temperature –0 °C to –20 °C

Product Description

Anti-Protein 4 is developed in goat using purified protein 4 as immunogen. The antibody is purified using Protein G chromatography. After purification the antibody preparation is conjugated to horseradish peroxidase.

Reagents

Anti-Protein 4 is provided as a solution at approximately 1 mg/ml in 0.01 M phosphate buffered saline, pH 7.4, containing 50% glycerol, 5 mM acetamidophenol, and 0.1% Microside III.

Storage

Store at –0 °C to –20 °C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Procedure

Buffers and Solutions

- Antibody Coating Buffer (0.05 M Carbonate/Bicarbonate, pH 9.6) – For 100 mL
  Dissolve one capsule of carbonate-bicarbonate buffer capsule (Product No. C 3041) in 100 mL of dH_2O.
- PBST buffer (0.01 M Sodium phosphate, 0.138 M Sodium chloride, 0.0027 M Potassium chloride, 0.05% TWEEN 20, pH 7.4) – For 1 Liter
  Dissolve one pouch of phosphate buffered saline with TWEEN 20 powder (Product No. P 3563) in 1 Liter of dH_2O.
- TMB ready-to-use peroxidase substrate solution (Product No. T 0440)
- 1 M Hydrochloric acid stop solution (Product Code 920-1)

Antibody Coating Procedure

1. Dilute Monoclonal Anti-Protein 4 (Product No. P 6242) to a concentration of 5 µg/ml in Antibody Coating Buffer. Dilute only enough antibody to coat the desired number of plates
2. Load 100 µl per well of the diluted Monoclonal Anti-Protein 4 (5 µg/ml) into a 96 well plate (Product No. M 4034).
3. Cover plate with sealing tape (Product No. T 2162) and allow to sit overnight at 4 °C.

Conjugate Preparation

Dilute stock Anti-Protein 4 - Peroxidase Conjugate (Product No. P 5867) to a final dilution of 1:3,000 in PBST buffer.

Plate Loading

1. Wash coated plate 3 times with PBST buffer before loading and pat dry.
2. Load 100 µl per well of diluted conjugate.
3. Load 50 µl per well of the positive and negative seed extracts in triplicate.
4. Cover plate with Mylar sealing tape (Product No. T 2162) and incubate at room temperature for 90 minutes.

Developing plate

1. Wash plate 3 times with PBST buffer and pat dry. This should be done quickly.
2. Add 100 µl of TMB substrate solution per well to the appropriate wells.
3. Develop plate for 10 minutes (± 30 seconds) at room temperature.
4. Add 100 µl of 1 M Hydrochloric acid stop solution per well to stop development. Mix by gently tapping the plate. Termination of the reaction is indicated by an even yellow color in all of the wells.

Data Generation

1. Wipe smudges from the bottom of the plate and read at 450 mm. The plate should be read within 1 hour of developing the plate.
2. Taking the average O.D. of the positive seed extract divided by the negative seed extract should generate the signal to noise ratio (S/N).

Product Profile

A suggested working dilution of 1:3,000 is determined for ELISA.

Using the above ELISA method, a dilution of 1:3,000 the Anti-Protein 4 -Peroxidase Conjugate (Product No. P 5867) will give an absorbance of approximately 1 OD with positive seed extract

Note: In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimal working dilutions by titration test.

This is a custom antibody preparation prepared specifically for Monsanto Corporation.

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