SouthernBiotech

SBA Clonotyping System-HRP

Cat. No.	Kit Format	Size
5300-05	Horseradish Peroxidase (HRP)	1.0 mL each

Description

The SBA Clonotyping System-HRP kit is designed for the isotyping of mouse monoclonal antibodies. It contains 2.5 mg of capture antibody and 1.0 mL of HRP conjugated anti-mouse Ig, mouse IgA, mouse IgG_1, mouse IgG_{2a}, mouse IgG_{2b}, mouse IgG_3, mouse IgM, mouse κ, mouse λ, and ABTS substrate. The kit may also be utilized for quantitative studies of mouse immunoglobulins in samples such as serum, supernatant, and ascites when used in conjunction with the Mouse Immunoglobulin Panel (SB Cat. No. 5300-01).

Applications

ELISA – Quality tested [1-42]

Kit Components

- Goat Anti-Mouse Ig, Human ads-UNLB
- Goat Anti-Mouse Ig, Human ads-HRP
- Goat Anti-Mouse IgA-HRP
- Goat Anti-Mouse IgG_1, Human ads-HRP
- Goat Anti-Mouse IgG_{2a}, Human ads-HRP
- Goat Anti-Mouse IgG_{2b}, Human ads-HRP
- Goat Anti-Mouse IgG_3, Human ads-HRP
- Goat Anti-Mouse IgM, Human ads-HRP
- Goat Anti-Mouse Kappa-HRP
- Goat Anti-Mouse Lambda-HRP
- ABTS Substrate Powder

Handling and Storage

- The purified (UNLB) antibody is supplied as 2.5 mg purified immunoglobulin in 1.0 mL of borate buffered saline, pH 8.2. *No preservatives or amine-containing buffer salts added*. Store at 2-8°C.
- The horseradish peroxidase (HRP) conjugates are supplied as 1.0 mL of stock solution in 50% glycerol/50% PBS, pH 7.4. No preservative added. Store at 2-8°C or long-term at -20°C.
- The ABTS substrate powder is supplied as 100 mg. Recommended storage is at 2-8°C. Protect from light.
- Reagents are stable for the period shown on the label if stored as directed.

Suggested Isotyping Protocol

- Dilute capture antibody to a concentration of 5 - 10 μg/mL in borate buffered saline (BBS), pH 8.2 or phosphate buffered saline (PBS), pH 7.4; add 0.1 mL to each well of the ELISA plate; alternatively, the antigen used for immunization may be used as the coating reagent
- Cover plate with a lid or plastic wrap and incubate in a humidified atmosphere at 2-8°C for a minimum of 12 hours
- Empty wells, wash 3X with BBS (or PBS) containing 0.05% Tween®, empty wells, and fill wells with BBS (or PBS) containing 1% bovine serum albumin (BBS/BSA)
- Allow antibody-coated plate to stand at room temperature for a minimum of 1 hour to block free binding sites on the plate
- Empty plate and wash 3X
- Add 0.1 mL of hybridoma supernatant to each well, cover plate, and incubate for 1 hour at room temperature with gentle shaking or overnight at 2-8°C
- Empty plate and wash 3X
- Dilute HRP-labeled detection antibody(ies) 1:250 – 1:500 in BBS/BSA, add 0.1 mL conjugate(s) to appropriate wells of the plate, cover plate, and incubate for 1 hour at room temperature with gentle shaking or overnight at 2-8°C
- Empty the plate and wash 5X
- Prepare ABTS substrate stock solution - Dissolve 15 mg ABTS powder in 1.0 mL of double glass-distilled water and store in the dark at 2-8°C; stable for approximately 4 weeks
- Prepare substrate solution - To 50 mL of double glass-distilled water, add 525 mg citric acid and stir to dissolve; adjust pH to 4.0 with 3N NaOH; to 10 mL of citrate substrate buffer, add 0.2 mL of ABTS stock solution and 10 μL of 30% H_2O_2
- Add 0.1 mL of substrate solution to each well of the plate
- Read optical density of each well at 405 nm after substrate addition

For Research Use Only. Not for Diagnostic or Therapeutic Use.

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