SouthernBiotech

SBA Clonotyping System-C57BL/6-AP

Cat. No.	Kit Format	Size
5300-04B	Alkaline Phosphatase (AP)	1.0 mL each

Description

The SBA Clonotyping System-C57BL/6-AP kit is designed for the isotyping of C57BL/6 mouse monoclonal antibodies. It contains 2.5 mg of capture antibody and 1.0 mL of AP conjugated anti-mouse Ig, mouse IgA, mouse IgG_1, mouse IgG_{2b}, mouse IgG_{2c}, mouse IgG_3, mouse IgM, mouse κ, mouse λ, and pNPP 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 C57BL/6 Mouse Immunoglobulin Panel (SB Cat. No. 5300-01B).

Applications

ELISA – Quality tested [1-4]

Kit Components

- Goat Anti-Mouse Ig, Human ads-UNLB
- Goat Anti-Mouse Ig, Human ads-AP
- Goat Anti-Mouse IgA-AP
- Goat Anti-Mouse IgG_1, Human ads-AP
- Goat Anti-Mouse IgG_{2b}, Human ads-AP
- Goat Anti-Mouse IgG_{2c}, Human ads-AP
- Goat Anti-Mouse IgG_3, Human ads-AP
- Goat Anti-Mouse IgM, Human ads-AP
- Goat Anti-Mouse Kappa-AP
- Goat Anti-Mouse Lambda-AP
- pNPP Substrate Tablets

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 alkaline phosphatase (AP) conjugates are supplied as 1.0 mL of stock solution in 50 mM Tris/1 mM $MgCl_2$/50% glycerol, pH 8.0, containing NaN_3 as preservative. Store at 2-8°C or long-term at -20°C.
- The pNPP substrate tablets are supplied as 20 x 5 mg. Store at 2-8°C. Protect from light.
- Reagents are stable for the period shown on the label if stored as directed.

Warning

Some reagents contain sodium azide. Please refer to product specific (M)SDS.

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 AP-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 substrate buffer - To 400 mL of double glass-distilled water, add 24.5 mg $MgCl_2 \cdot 6H_2O$ and 48 mL diethanolamine; adjust pH to 9.8 with 5N HCl and make up to 500 mL with distilled water
- Prepare a 1 mg/mL substrate solution (e.g., one 5 mg tablet + 5 mL substrate buffer) and add 0.1 mL 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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References

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