

SBA Clonotyping™ System/AP

Cat. No.	Form	Quantity
5300-04	Kit	1

DESCRIPTION

Assay: Alkaline phosphatase (AP)-based Enzyme-Linked-Immunosorbent-Assay (ELISA) and immunoblotting.

- Components:**
- 1 mL goat anti-mouse Ig capture antibody (2.5 mg/mL)
 - 1 mL AP-labeled goat anti-mouse Ig screening antibody
 - 1 mL AP-labeled goat anti-mouse IgM
 - 1 mL AP-labeled goat anti-mouse IgG₁
 - 1 mL AP-labeled goat anti-mouse IgG_{2a}
 - 1 mL AP-labeled goat anti-mouse IgG_{2b}
 - 1 mL AP-labeled goat anti-mouse IgG₃
 - 1 mL AP-labeled goat anti-mouse IgA
 - 1 mL AP-labeled goat anti-mouse κ
 - 1 mL AP-labeled goat anti-mouse λ
 - p-nitrophenyl phosphate substrate tablets (20 x 5 mg)

RESEARCH APPLICATIONS

- Initial screening of fusions and selection of antibody-producing hybridomas
- Identification of the heavy and light chain isotypes of antibodies
- Selection and isolation of isotype switch variants

CHARACTERIZATION

To ensure lot-to-lot consistency, each batch of product is tested by ELISA for conformance to characteristics of a standard reference reagent.

WORKING DILUTIONS

ELISA (AP): 1:250-1:500

Immunoblotting: Since immunoblotting methods vary, you should determine the optimum working dilution of the product that is appropriate for your specific need.

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

RECOMMENDED ELISA PROCEDURE FOR ISOTYPE DETERMINATION

(NOTE: ELISA PLATE FORMATS SHOULD BE DESIGNED BY THE INVESTIGATOR)

- 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.
- Dilute capture antibody to a concentration of 5-10 $\mu g/mL$ in 100 mM borate buffered saline (BBS), pH 8.0 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 the 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) in BBS/BSA, add 0.1 mL conjugate(s) to appropriate wells of the plate, cover the 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 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 at 10 minutes and 20 minutes after substrate addition.
- Record data.

HANDLING AND STORAGE

- The purified (UNLB) antibody is supplied 100 mM 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 in a stock solution in 50mM Tris/1mM $MgCl_2$ /50% Glycerol, pH 8.0, containing 0.1% NaN_3 as preservative. Store at 2-8°C.
- All system components can be stored for several months at 2-8°C without appreciable loss of activity. For long-term storage, the purified antibody and AP conjugates should be aliquoted undiluted and stores at -20°C.

WARNING

Reagents contain sodium azide which is very toxic if ingested or inhaled. Avoid contact with skin, eyes, or clothing. Wear eye or face protection when handling. If skin or eye contact occurs, wash with copious amounts of water. If ingested or inhaled, contact a physician immediately. Sodium azide yields toxic hydrazoic acid under acidic conditions. Dilute azide-containing compounds in running water before discarding to avoid accumulation of potentially explosive deposits in lead or copper plumbing.