Mouse Antibody Isotyping Kit

Catalog Number
AKR-151  10 assays
**Introduction**

Determining the class and subclass of a monoclonal antibody is useful in determining the best immunoglobulin purification method. For example, IgA and IgM are often best purified by size (gel exclusion) or on immuno affinity separation columns, whereas IgG₂a and IgG₂b can be purified on protein A at a pH of 7 to 8. IgG₁ binds best to protein A at a pH of 8 to 9. In addition, each class and isotype can be digested to Fab fragments using the appropriate amount of pepsin or other enzymes.

The Mouse Antibody Isotyping Kit is a rapid lateral flow assay with ELISA sensitivity for monoclonal antibody class and subclass determination. The assay can be run on both tissue culture supernatant fluid and on mouse ascites fluid. Results are read in 5 to 10 minutes.

**Assay Principle**

Each pouch contains two cassettes; one cassette detects IgG₁, IgG₂a, and IgG₂b, and the other cassette detects IgG₃, IgA, and IgM.

When a properly diluted sample containing a specific isotype is added to the sample-well, specific-class and subclass soluble complexes are formed with the embedded gold conjugates. These complexes travel the length of the membrane and are resolved on the anti-isotype and class-specific antibody-impregnated membrane. A control line will appear on the membrane in the region marked “C” on each cassette to indicate a successful run.

**Related Products**

1. AKR-152: Mouse Antibody Isotyping Kit with Kappa & Lambda Light Chains
2. AKR-110: Rapid GST Inclusion Body Solubilization and Renaturation Kit
3. AKR-130: His-Tag ELISA Kit

**Kit Components**

1. Antibody Isotyping Detection Cassettes (Part No. 215001): 10 pouches, 2 cassettes per pouch
2. Sample Diluent (Part No. 215101): One 45 mL bottle

**Materials Not Supplied**

1. Antibody samples: ascites fluid or cell culture supernatant
2. Vortex mixer

**Storage**

Store kit components at room temperature until their expiration dates.
Assay Protocol

I. Testing Ascites Fluid
1. Dilute ascites samples 1:8000 by adding 0.5 µL of ascites fluid to 4 mL of Sample Diluent. Vortex to mix.
2. Remove both cassettes from a single pouch. One cassette detects IgG1, IgG2a, and IgG2b, and the other cassette detects IgG3, IgA, and IgM.
3. Add 150 µL of diluted sample to the sample well at the bottom of one cassette (marked on cassette with “S”). Repeat with the second cassette.
4. Incubate at room temperature for 5-10 minutes.
   *Note: Do not incubate for more than 10 minutes.*
5. Inspect both cassettes. The darkest red line indicates the antibody isotype. In some cases weaker lines may appear, indicating host serum immunoglobulins may be present in the ascites fluid. A red line should also appear in the control area marked “C” to ensure the test was run correctly.

II. Testing Cell Culture Supernatants
1. Dilute cell culture supernatant samples as follows:
   a. For samples containing antibody concentrations ≥1 µg/mL, dilute samples 1:100 by adding 5 µL of cell culture supernatant to 0.5 mL of Sample Diluent. Vortex to mix.
   b. For samples containing antibody concentrations <1 µg/mL, dilute samples 1:10 by adding 55 µL of cell culture supernatant to 0.5 mL of Sample Diluent. Vortex to mix.
2. Remove both cassettes from a single pouch. One cassette detects IgG1, IgG2a, and IgG2b, and the other cassette detects IgG3, IgA, and IgM.
3. Add 150 µL of diluted sample to the sample well at the bottom of one cassette (marked on cassette with “S”). Repeat with the second cassette.
4. Incubate at room temperature for 5-10 minutes.
   *Note: Do not incubate for more than 10 minutes.*
5. Inspect both cassettes. The darkest red line indicates the antibody isotype. Occasionally one or more weak red lines may indicate multiple hybridoma clones. A red line should also appear in the control area marked “C” to ensure the test was run correctly.

**Example of Results**
The following figures demonstrate typical Mouse Antibody Isotyping Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

![Figure 1: Mouse Antibody Isotyping Results from Two Different Samples. Left: Dark red line indicates isotype IgA. Right: Dark red line indicates isotype IgG\(_{2a}\).](image)

**Warranty**
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