



Rapid Mouse Monoclonal Antibody Isotyping Kit (Cat#: ISO-M6-5, -10, -20)

Lateral flow assay for 5 minute determination of 4 mouse Ig heavy chain isotypes and 2 light chain subclasses

The Rapid Mouse Monoclonal Antibody Isotyping Kit is a five-minute rapid lateral flow assay with ELISA sensitivity for monoclonal antibody class and subclass determination. Acceptable sample types include hybridoma culture supernatant and purified monoclonal antibody. Ascites fluid is not recommended since it normally contains multiple immunoglobulins. However, this kit can still be used to determine the relative distribution of the main isotypes in a sample of ascites fluid.

Assay Utility

Antibodies of different isotypes and subclasses usually demonstrate different effector functions in vivo. Therefore, when screening hybridomas, it is necessary to focus on the most high-producing clones with the desirable subclass and isotype.

In addition, determining the class and subclass of a monoclonal antibody is useful in choosing the best immunoglobulin purification method. For example, IgA and IgM are often best purified by size (gel exclusion) or on immunoaffinity separation columns, whereas IgG2a and IgG2b can be purified on Protein A at pH of 7 to 8. IgG1 binds best to Protein A at pH of 8 to 9.

Assay Background Information

When a sample containing mIg of a specific isotype is added to the sample well, specific class and subclass soluble complexes are formed with embedded goat anti-mouse gold conjugates. These complexes travel the length of the membrane, and are resolved and

captured at different regions of the NC membrane impregnated with isotype and class-specific antibodies. A control line will appear on the membrane at the region marked "C", where polyclonal donkey anti-goat Ig is impregnated, indicating a successful run.

Sample Requirements and Dilution Conditions

Cell Culture Supernatant	Purified Antibody	Ascites
1:100 dilution with PBS. Can also be run neat, without dilution.	Dilute samples to 0.5 - 2 µg/mL in PBS.	Ascites fluid is not recommended since it normally contains multiple Igs. However, this kit can still be used to determine the relative distribution of the main isotypes in a sample of ascites fluid. The recommended dilution is 1:10,000.

Kit Components

The isotyping kit has three different sizes: ISO-M6-5, ISO-M6-10, and ISO-M6-20 come with 5, 10 or 20 pouches containing two cassettes per pouch: one cassette is for determining IgG1, IgG2a, and IgG2b, and the other cassette is for determining Kappa, Lambda and IgM. Both strips contain a control line.

Protocol

The manual should be read in its entirety before making any test. Prior to use, return the product to room temperature.

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NOTE: Prepare test samples before removing the strips from the sealed dry bag.

NOTE: Allow strips to come to room temperature before they are removed from sealed bag. Promptly return unused strips in their original bag and keep in 4°C.

Protocol

- 1) Place the cassettes in horizontal plane.
- 2) Add 80-100 μ L antibody liquid directly to the Sample Well. Wait for 5 minutes.
- 3) A red test line at the location corresponding to antibody-specific isotype and a red control line at "C" position will appear within 1-5 minutes.

NOTE: Do not read after 20 minutes.

NOTE: If the color of tissue culture medium causes high background, 40-60 μ L PBS can be chased to clear the background.

Result Interpretation

1. Invalid result: no red line at "C".
2. Negative result: only one red line at "C".
3. Positive results:
 - a). Single monoclonal antibody: In addition to the positive control "C" line, only one heavy chain and one light chain will appear.
 - b). Multiple monoclonal antibodies: In addition to the positive control line, more than one heavy chain and/or one light chain will appear. The line intensity can be used to judge the dominant isotypes.
 - c). Ascites fluid: the darker red line indicates the class or subclass present. Often times, additional weaker red lines appear, indicating the presence of host serum immunoglobulins in the ascites.

Examples and Sensitivities



PBS 0.1 0.2 0.5 1.0 μ g/mL



PBS 0.1 0.2 0.5 1.0 μ g/mL

Performance

Minimum detectable concentration is 100 ng/mL. There is no cross-reactivity among each isotype.

Storage

Store the strips in their original packaging at room temperature. This product is stable for up to 18 months from the certified date. Freezing the strips is not recommended.