

Rapid Mouse Isotyping Kit – Gold Series

Lateral flow assay for ten minute determination of 8 Mouse
Immunoglobulin Isotypes and 2 light chain subclasses

Patent Pending Technology

User Manual (Version Feb 2013)

**Cat #: LFM-ISO-1-5
LFM-ISO-1-10**



**We Provide You With Excellent
Protein Array Systems and Service**

**Tel:(Toll Free) 1-888-494-8555 or 770-729-2992; Fax: 1-888-547-0580;
Website:www.raybiotech.com Email: info@raybiotech.com**

【Intended Use】

This product uses lateral flow technology to quickly determine the mouse monoclonal antibody heavy chain isotypes and light chain subclasses in 10 minutes. 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. The kit consists of three groups of test strips. The first group (Strip 1; Red) is for mouse IgG1 isotype and the two light chain subclasses Kappa and Lambda (IgG1/Kappa/Lambda); the second group (Strip 2; Green) is for mouse IgG isotypes (IgG1/IgG2a/IgG2b /IgG3); and the third group (Strip 3; Yellow) is for other mouse isotypes (IgA / IgD / IgE /IgM), see Figure 1. Altogether, these strips can be used to determine the unknown mouse monoclonal antibody heavy chain (IgG1, IgG2a, IgG2b, IgG3, IgA, IgM, IgD, IgE) and light chain (Kappa, Lambda).

【Principle】

Mouse isotyping specific capture antibodies are coated on nitrocellulose membranes in three different test strips. A pan detection antibody which recognizes all the mouse isotypes is labeled with colloidal gold and coated on the conjugation pad on the same strips. After sample is loaded in the sample pad, it passes through the conjugation pad, capture antibody coating membrane and moves toward the other end of the strip through lateral flow chromatography. A complex is formed first by the antibody detected in the sample and the colloidal gold-labeled detection antibody. This complex moves further and is captured by different coated capture antibodies in that location. The aggregation of the complex (capture antibody - mouse monoclonal antibody - gold-labeled detection antibody) on site produces the visible red lines. If it is a negative sample, the double antibody sandwich complex cannot be formed in the region of the detection line, and no significant color line appears. Regardless of whether mouse monoclonal antibody is present in the test sample or not, a red line will appear in the control line area for internal control of the reagents. This color band appears in the control line region is to determine whether there is sufficient specimens and whether the chromatography process is normal.

【Kit Components】

This kit contains three sealed packaging bags. Each bag comprises five (ten) test strips of the following three groups (Figure 1), which is enough for testing 5 (10) samples.

1. Strip 1 (Red): IgG1 and light chain (κ , λ) isotyping strip
2. Strip 2 (Green): IgG (IgG1/IgG2a,/IgG2b/IgG3) isotyping strip
3. Strip 3 (Yellow): IgA, IgD, IgE, IgM isotyping strip

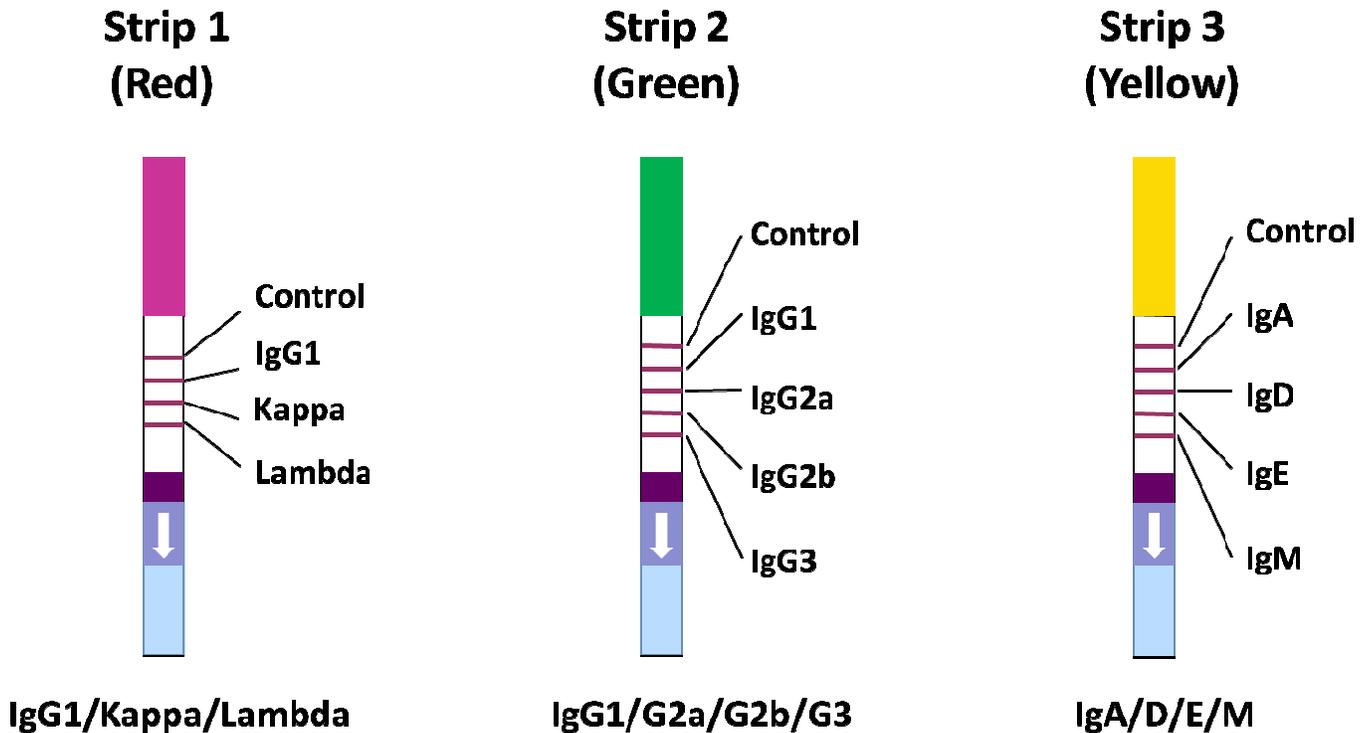


Figure 1. Strip diagrams

【Sample Requirements】

Sample requirement and dilution conditions

Cell Culture Supernatant	Purified Antibody	Ascites
Can 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 immunoglobulins. 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

【Protocol】

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

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.*

NOTE: *Suggested strip testing sequence: Use Strip 1 first; if the unknown antibody is not IgG1 isotype, then use Strip 2. If the sample shows no discernable isotype on Strip 2, Strip 3 should be employed. All three strips may be run in parallel to insure the quickest path to isotype determination.*

1. Add 40µl antibody liquid sample onto the sample pad indicated by the arrows printed on the strips (see Strip 1 picture in Results Interpretation section of this manual). Alternatively, the strip sample pad can be directly dipped into the hybridoma culture supernatant. Please note that the culture

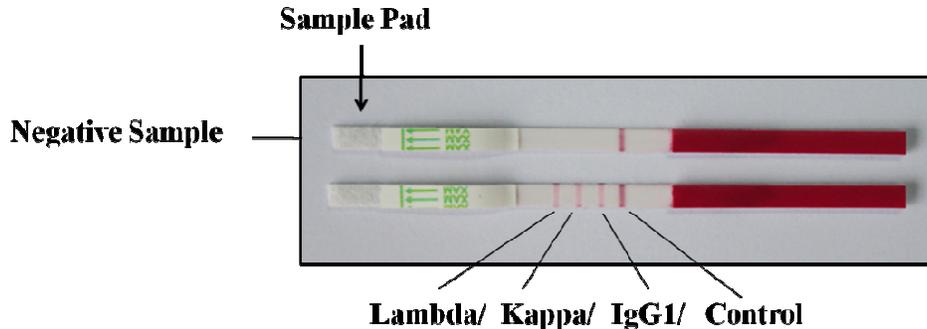
fluid shouldn't come into direct contact with the area beyond the arrow front position. After ten minutes, remove the strip from the culture fluid and place on a flat and clean surface.

2. A red test line and a red control line will appear at the corresponding locations of the test strip within 10 minutes; Wait for the appearance of the red bands then read the result by making a comparison with the representative pictures in the Results Interpretation section of this manual. Results should be read within 12-30 minutes.

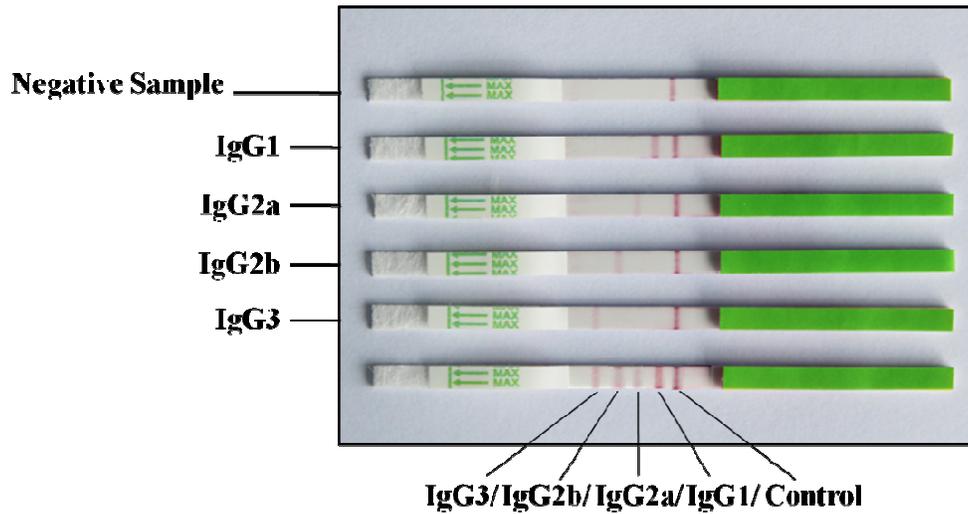
【Result Interpretation】

1. Invalid result: no red band appeared in the control line region.
2. Negative result: only a red band appeared in the control line region;
3. Positive results:
 - a. Pure monoclonal antibody: In addition to the positive control line, only one heavy chain and one light chain will appear.
 - b. Unpurified monoclonal antibody: 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.

Representative Strip Pictures

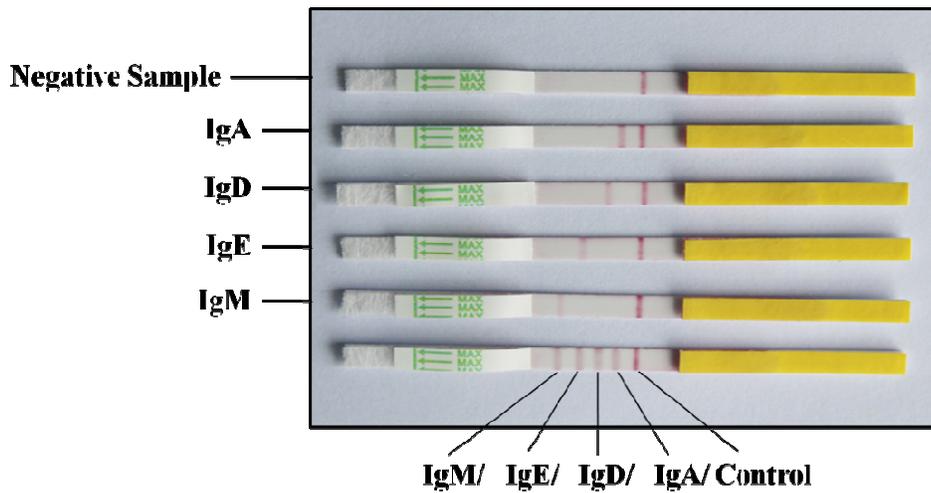


**Strip 1 (Red): Mouse IgG1 and light chain
(IgG1/Kappa/Lambda) isotyping strip**



Strip 2 (Green)

Mouse IgG (IgG1/IgG2a/IgG2b /IgG3) isotyping strip



Strip 3 (Yellow)

Mouse IgA / IgD / IgE / IgM isotyping strip

【Performance】

1. Minimum detectable concentration is 100 ng/mL. There is no cross-reactivity among each isotype.
2. The RayBiotech Rapid Mouse Isotyping Kit Gold Series offers a more comprehensive isotype detection than other similar products in the market, detecting all the heavy chains (IgG1, IgG2a, IgG2b, IgG3, IgA, IgM, IgD, and IgE) and light chains (Kappa & Lambda).
3. The RayBiotech Rapid Mouse Isotyping strips are economically designed. Since IgG1 is the dominant heavy chain isotype, Strip 1 can in some cases be used as a stand-alone test to determine both heavy and light chain subtypes, reducing both cost and work load. Strip 1 is sold separately as **Rapid Mouse Isotyping Strip 1 – Gold Series (LFM-ISO-S1)**.

【Storage】

- ❖ Store the strips in their original packaging at 4 °C. This product is stable for up to 12 months from the certified date.
- ❖ Bring strips to room temperature before use and promptly return unused strips in their original bag and store at 4 °C.
- ❖ Freezing the strips is not recommended.