Iso-Gold™ Rapid Mouse-Monoclonal Isotyping Kit (Cat. #: ISOT-001)

The Iso-Gold™ Rapid-Mouse-Mononclonal Isotyping Kit is a 5 (five)-minute 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.

Assay Utility:
Determining the class and subclass of a mononclonal 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 IgG_{2a} and IgG_{2b} can be purified on protein A at a pH of 7 to 8. IgG_1 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.

Assay background information:
There are two (2) cassettes in each of the ten (10) pouches: one cassette for detecting IgG_1, IgG_{2a}, and IgG_{2b}; the other cassette detects IgG_3, 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 on the cassette marked "C", indicating a successful run.

Typically, when antibodies are tested at ten (10) nanograms per milliliter, results are read at five (5) to ten (10) minutes. (Results should not be read *after* ten (10) minutes.)

Monoclonal antibody ascites fluid:
For **ascites fluid**, the darker red line indicates the class or subclass present. Often, additional weaker red lines appear indicating the presence of host serum immunoglobulins in the ascites.

Procedure for ascites fluid:
1) Dilute ascites 1:8000 by adding 0.5µl of ascites fluid to 4 ml of Sample Diluent - **vortex to mix**
2) Add 150 µl of diluted ascites fluid to the Sample Well (S).
3) Wait 5 (five) minutes
4) Read results – the darker line is the isotype

NOTE: **Do not read after ten (10) minutes**

Cell culture/supernatant fluid:
For **cell culture**/**supernatant fluid** a dark red line indicates which isotype or class-specific antibody is present. In very few instances, additional weak red lines may appear indicating multiple hybridoma clones.

Procedure for cell culture/supernatant fluid:
1) Dilute cell culture/supernatant fluid 1:100 by adding 5.0 µl of supernatant fluid to 0.5 ml of Sample Diluent - **vortex to mix**
2) Add 150 µl of diluted supernatant fluid to Sample Well (S)
3) Wait 5 (five) minutes
4) Read results

NOTE: Do not read after ten (10) minutes

Note: For supernatants that contain the monoclonal antibody at less than 1 microgram per ml, dilute the sample 1:10 as follows:
- Add 55 µl of supernatant to 500 µl of sample diluent - **vortex to mix.**
- Repeat Steps **2** through **4**

Kit Components:
• Ten (10) pouches containing two (2) cassettes per pouch; one cassette contains an anti-IgG_1, anti-IgG_{2a}, and anti-IgG_{2b} isotype impregnated strip; and the other cassette contains an anti-IgG_3, and anti-IgA, anti-IgM isotype impregnated strip. Both strips contain a control line.
• Sample Diluent - 45 ml

Note: Store kit and components at room temperature – Shelf life 18 months.