Isotyping Kit: Rapid Mouse Monoclonal Isotyping Kit Iso*Max*™

10 Test Pack

Catalog # KDA-010

I. Overview

Rockland Immunochemicals **Iso*Max***™ Rapid Mouse Monoclonal Isotyping Kit is a rapid (5 minute) lateral flow assay with sensitivity equal to ELISA for monoclonal antibody class and subclass determination. The assay is designed to test antibody present in tissue culture supernatant or mouse ascites fluid. This kit contains 10 packs of 2 cassettes sufficient for testing up to 10 samples.

Please read the entire product insert prior to use.

II. Kit Principle

Each kit contains 10 packs of 2 cassettes each: one cassette for detecting IgG_1, IgG_{2a} and IgG_2b; the other cassette for detecting IgG_3, IgA and IgM. When a properly diluted sample containing a specific isotype antibody is added to the sample well, a specific class and subclass soluble complex is formed with the embedded gold conjugates. These complexes travel the length of the membrane and are resolved on the anti-isotype and anti-class-specific antibody impregnated membrane. A control line will appear on the membrane in the region marked "C" on the cassette indicating a successful run (positive internal control).

Typically when antibodies are applied at a concentration of 10 ng/mL the best results are obtained by reading the cassette 5 to 10 minutes after application. Results should NOT be read after 10 minutes.

III. Intended Use

Use Rockland Immunochemicals **Iso*Max***™ Rapid Mouse Monoclonal Isotyping Kit for determining the class and subclass of a monoclonal antibody. Isotype determination is a prerequisite for selecting the best immunoglobulin purification method. For example, IgA and IgM are often best purified by size (gel filtration) or on immunoaffinity support columns, whereas IgG_{2a} and IgG_{2b} can be purified by protein A chromatography at a pH of 7 to 8. IgG_1 binds best to protein A at a pH of 8 to 9. Isotype determination is also required if an antibody is to be digested by pepsin or papain as each isotype shows varying sensitivity to enzymatic digestion. If you require additional assistance please call or e-mail our technical service representatives at 800-656-7625 or tech@rockland-inc.com.

IV. Storage and Stability

This kit is stable for at least 18 months when stored at +20 °C.

V. Number of Assays

Components in this kit are sufficient to run 10 isotype class and subclass determinations.

VI. Kit Advantages

Rockland Immunochemicals **Iso*Max***™ Rapid Mouse Monoclonal Isotyping Kit is best for isotype and purity determinations for mouse monoclonal antibodies. In 5 minutes you can:

- ***Determine the isotype of your monoclonal antibody.*** Both class and subclass determination.
- ***Assess the purity of your purified monoclonal antibody.*** Single line indicates purity.
- ***Verify the purity of your purchased monoclonal antibody.*** Multiple lines indicate contamination.
- ***Save valuable time.*** By reducing the time for traditional ELISA procedures from 4 to 6 hours to just 5 minutes.

VII. Comparative Study

We compared the monoclonal isotyping results of an ELISA-format isotyping assay (figure 1 and 2) and the **Iso*Max***™ isotyping kit (figure 3). The results demonstrate outstanding correlation between these two assay formats. The ELISA required controls, an ELISA plate and substrate and took 8 hours of hands-on time to coat the plates, block and subsequently perform the assay using 12 different antibodies. The **Iso*Max***™ isotyping kit pipeting time was 10 minutes and all 20 assays were run simultaneously. Each cassette was read at 5 minutes, therefore the total hands-on time, including scoring of the results, was under twenty minutes.

Figure 1. Comparative ELISA format assay.

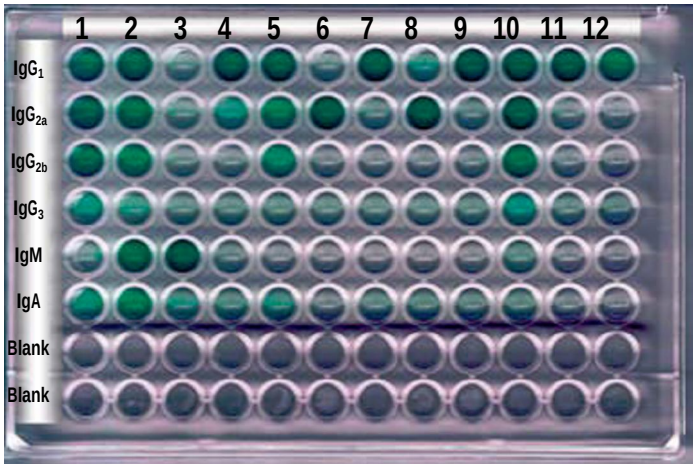

Figure 2. Tabulation of Comparative ELISA format assay data.

Anti-	mAb	1	2	3	4	5	6	7	8	9	10	11	12
IgG_1	A	0.722	0.788	0.099	0.899	0.787	0.085	0.917	0.218	0.850	0.692	0.810	0.593
IgG_{2a}	B	0.931	0.663	0.091	0.235	0.419	0.931	0.074	1.075	0.079	0.859	0.073	0.069
IgG_{2b}	C	0.665	0.460	0.075	0.062	0.371	0.063	0.072	0.073	0.065	0.456	0.062	0.064
IgG_3	D	0.209	0.134	0.094	0.088	0.108	0.085	0.085	0.088	0.083	0.165	0.084	0.089
IgM	E	0.158	0.739	0.853	0.089	0.069	0.056	0.053	0.057	0.054	0.186	0.061	0.069
IgA	F	0.302	0.409	0.116	0.106	0.181	0.060	0.085	0.095	0.089	0.117	0.085	0.076
Kappa	G	0.941	0.873	0.710	0.817	0.777	0.618	0.767	0.753	0.781	0.868	0.490	0.564
Lambda	H	0.249	0.209	0.107	0.098	0.109	0.085	0.090	0.09	0.084	0.154	0.255	0.084

The data shows excellent correlation between the ELISA-format isotyping assay and the **Iso*Max***™ isotyping kit. In addition to the correct interpretation of the isotype, the presence of contaminating antibody present in each monoclonal can be accurately determined with the **Iso*Max***™ isotyping kit. Impurities in the form of contaminating isotypes and subclasses correlate well the ELISA data. This demonstrates that the **Iso*Max***™ isotyping kit provides the added feature of determining the purity of the monoclonal being tested.

Figure 3. Iso*Max*™ **isotyping kit cassette results.**

VIII. Kit Components

1. (10) packs containing 2 cassettes per pack. One cassette with a test strip impregnated with anti-IgG_1, anti-IgG_{2a} and anti-IgG_{2b} specific antibodies; and one cassette with a test strip impregnated with anti-IgG_3, anti-IgA and anti-IgM specific antibodies.
2. (1) x 45 mL Sample Diluent
3. Instruction Manual

IX. Isotyping Method

A. For mouse monoclonal antibody present in ascites fluid:

1. For ascites fluid the darker red line indicates the class or subclass of antibody present. Often an additional weaker red line may appear indicating the presence of host (endogenous) serum immunoglobulins present in the ascites.
2. Dilute ascites 1:8,000 by adding 0.5 µL of ascites fluid to 4 mL of Sample Diluent. Thoroughly mix the sample.
3. Add 150 µL of diluted ascites fluid to each of the sample wells.
4. Wait 5 minutes.
5. Read the results - the darker line is the isotype of the monoclonal antibody present in the ascites.
6. Do not read after 10 minutes.
7. Cassettes may be scanned or photographed for a permanent record of results.

B. For mouse monoclonal antibody present in cell culture supernatant fluid:

1. For cell culture supernatants a dark red line indicates the class or subclass of antibody present. If one or more additional weaker bands appear, then the antibody may be contaminated by multiple hybridoma clones.
2. Dilute the cell culture supernatant fluid 1:100 by adding 5.0 µL of cell culture supernatant fluid to 0.5 mL of Sample Diluent[1]. Vortex to thoroughly mix the sample.
3. Add 150 µL of diluted cell culture supernatant fluid to each of the sample wells.
4. Wait 5 minutes.
5. Read the results - the dark line is the isotype of the monoclonal antibody present in the cell culture supernatant fluid.
6. Do not read after 10 minutes.
7. Cassettes may be scanned or photographed for a permanent record of results.

[1] Dilute the sample 1:10 for supernatants that contain monoclonal antibody at less than 1.0 µg/mL by adding 55 µL of cell culture supernatant to 500 µL of Sample Diluent. Mix thoroughly and continue with Step 3.

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XI. Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 326, Gilbertsville, Pennsylvania, USA.

XVII. Additional Products and Services

Additional Products	Code	Size	Price
Iso*Max*™ Rapid Mouse Monoclonal Isotyping Kit	KDA-005	1 X 5 pack	www.rockland-inc.com
Iso*Max*™ Rapid Mouse Monoclonal Isotyping Kit	KDA-010	1 X 10 pack	www.rockland-inc.com
Custom Monoclonal Antibody Development	MAB-001	1 each	*inquire*
Roller Bottle Cell Culture for Antibody Production	MAB-062	1 each	*inquire*
Custom Mouse Ascites (Balb/c) for Antibody Production	CUST25	1 each	*inquire*
SEPHAROSE™ PROTEIN A	PA50-00-0005	5 ml	www.rockland-inc.com
SEPHAROSE™ PROTEIN A	PA50-00-0025	25 ml	www.rockland-inc.com

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