# Rapid Mouse Monoclonal Isotyping Kit IsoMax™ Protocol
# KDA-005

## I. Kit Components

| Component | |
|---|---|
| KDA-001 | |
| | (5) pack containing 2 cassettes per pack. One cassette with a test strip impregnated with anti-IgG1, anti-IgG2a, and anti-IgG2b specific antibodies: and one cassette with a test strip impregnated with anti-IgG3, anti-IgA, and anti-IgM specific antibodies. |
| KDA-002 | |
| | (1) x 25 mL sample diluent |
| Instruction Manual | |
| | Please read the entire product insert prior to use. |

## II. Overview

Rockland **IsoMax**™ Rapid Mouse Monoclonal Isotyping Kit is a rapid (5 minute) lateral flow assay with sensitivity equal to ELISA (Enzyme Linked Immunosorbent Assay) for monoclonal antibody class and subclass determination. The assay is designed to test antibody present in tissue culture supernatant or mouse ascites fluid.

## III. Kit Principle

Each kit contains 5 packs of 2 cassettes each: one cassette for detecting anti-IgG1, anti-IgG2a, and anti-IgG2b; the other for detecting anti-IgG3, anti-IgA, and anti-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 10ng/mL the best results are obtained by reading the cassettes 5 to 10 minutes after application. Results should **NOT** be read after 10 minutes.

## IV. Intended Use

Use Rockland **IsoMax**™ 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 IgG2a and IgG2b can be purified by protein A chromatography at a pH of 7 to 8. IgG1 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 of papain as each isotype shows varying sensitivity to enzymatic digestion. If you require additional assistance, please call or email our technical service representatives at 800-656-7625 or tech@rockland.com.

## V. Storage and Stability

This kit is stable for up to 24 months when stored at 2-8°C. Refer to kit for specific expiration dates.

## VI. Number of Assays

Components in this kit are sufficient to run 5 isotype class and subclass determinations.

## VII. Kit Advantages

Rockland **IsoMax**™ 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 4-6 hours to just 5 minutes.

## VIII. Comparative Study

We compared the monoclonal isotyping results of an ELISA-format isotyping assay (figure 1 and 2) and the **IsoMax**™ Isotyping kit (figure 3). The results demonstrate an 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 **IsoMax**™ Isotyping kit pipetting time was 10 minutes and all 20 assays we run simultaneously. Each cassette was read at 5 minutes, therefore the total hands-on time, including scoring of the results, was under 20 minutes.

**Figure 1. Comparative ELISA format assay.**

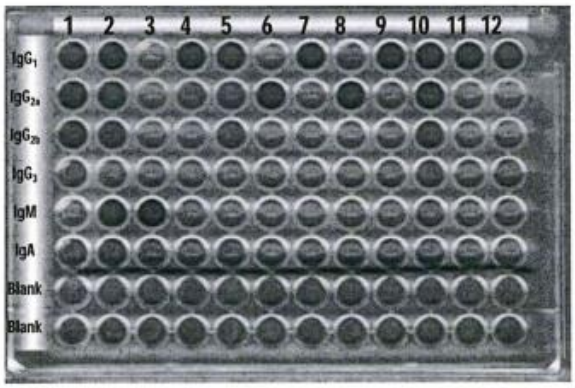


**Figure 2. Tabulation of Comparative ELISA format assay data.**

| 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 **IsoMax**™ Isotyping kit. In addition to the correct interpretation of the isotype, the presence of the contamination antibody present in each monoclonal can be accurately determined with the **IsoMax**™ Isotyping kit. Impurities in the form of the contaminating isotypes and subclasses correlate with the ELISA data. This demonstrates that the **IsoMax**™ Isotyping kit provides the added feature of determining the purity of the monoclonal being tested.

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


## 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 0.5µL of ascites fluid to 4µL of Sample Diluent. Thoroughly mix sample.
3. Add 150µL 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 permanent record of results.

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

1. For cell culture supernatants the darker 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 5.0µL of cell culture supernatant fluid to 0.5µL of Sample Diluent. Vortex to thoroughly mix the sample.

   Note: Dilute the sample 1:10 for supernatants that contain monoclonal antibody at less than 1.0µg/mL by adding 55µL of cell culture supernatant to 500µL of Sample Diluent. Mix thoroughly.
3. Add 150µL of diluted cell culture supernatant 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 cell culture supernatant fluid.
6. Do not read after 10 minutes.
7. Cassettes may be scanned or photographed for permanent record of results.

## X. Trademarks

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## XI. Disclaimer

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## XII. Additional Products

| Additional Products | p/n | Size |
|---|---|---|
| **IsoMax**™ Rapid Mouse Monoclonal Isotyping Kit | KDA-005 | 1x5 pack |
| **IsoMax**™ Rapid Mouse Monoclonal Isotyping Kit | KDA-010 | 1x10 pack |
| Custom Monoclonal Antibody Development | MAB-001 | 1 Each |
| Roller Bottle Cell Culture for Antibody Production | MAB-062 | 1 Each |
| Custom Mouse Ascites (Balb/c) for Antibody Production | CUST25 | 1 Each |
| SEPHAROSE™ Protein A | PA50-00-0005 | 5mL |
| SEPHAROSE™ Protein A | PA50-00-0025 | 25mL |
| SEPHAROSE™ Protein G | PG50-00-0002 | 2mL |

# Iso-Gold™ Rapid Mouse-Monoclonal Isotyping Kit (Cat. #: ISOT02-010)

The Iso-Gold™ Rapid Mouse-Monoclonal 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 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 $IgG_{2a}$ 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 Buffer - **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 Buffer - **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** above.

**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-$IgG_3$, anti-IgA, and anti-IgM isotype impregnated strip. Both strips contain a control line.
- Sample Diluent Buffer - 45 ml

**Note:** Store Sample Diluent Buffer at 2-8°C. The other kit components may be stored at room temperature. If the entire kit is stored at 2-8°C, make sure the cassettes are sealed in the desiccated pouch and allow them to come to room temperature before opening the pouch.

– Shelf-life 24 months from manufacturing date.



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