



# Aviva Rapid Mouse Monoclonal Isotyping Kit

## 5 Test Pack

Catalog # OKRA00053

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### I. Overview

Aviva 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 5 packs of 2 cassettes sufficient for testing up to 5 samples.

**Please read the entire product insert prior to use.**

### II. Kit Principle

Each kit contains 5 packs of 2 cassettes each: one cassette for detecting IgG<sub>1</sub>, IgG<sub>2a</sub> and IgG<sub>2b</sub>; the other cassette for detecting IgG<sub>3</sub>, 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 Aviva 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<sub>2a</sub> and IgG<sub>2b</sub> can be purified by protein A chromatography at a pH of 7 to 8. IgG<sub>1</sub> 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 1-888-880-0001 or [techsupport@avivasysbio.com](mailto:techsupport@avivasysbio.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 5 isotype class and subclass determinations.

### VI. Kit Advantages

Aviva Rapid Mouse Monoclonal Isotyping Kit is best for isotype and purity determinations for mouse monoclonal antibodies. In 5 minutes you can:

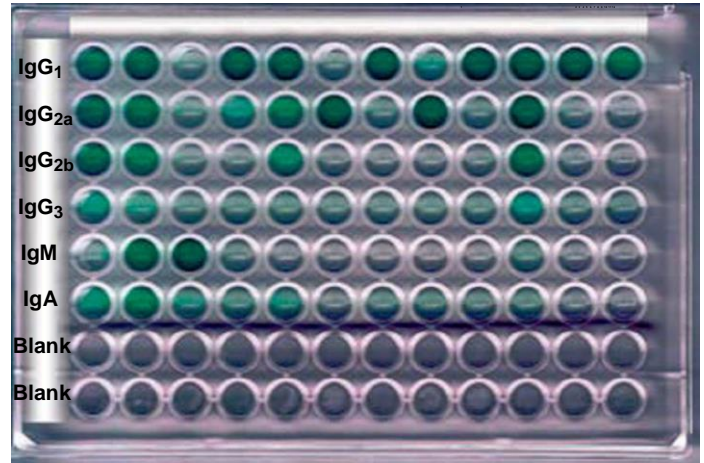
**Determine the isotype of your monoclonal antibody.** Both class and subclass determination.

**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.

Figure 1. Comparative ELISA format assay.



**VII. Comparative Study**

We compared the monoclonal isotyping results of an ELISA-format isotyping assay (figure 1 and 2) and the **Aviva 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 **Aviva 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 2. Tabulation of Comparative ELISA format assay data.

Anti-	mAb	1	2	3	4	5	6	7	8	9	10	11	12
IgG <sub>1</sub>	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 <sub>2a</sub>	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 <sub>2b</sub>	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 <sub>3</sub>	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 **Aviva 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 **Aviva Isotyping Kit**. Impurities in the form of contaminating isotypes and subclasses correlate well the ELISA data. This demonstrates that the **Aviva Isotyping Kit** provides the added feature of determining the purity of the monoclonal being tested.

Figure 3. Aviva isotyping kit cassette results.



### VIII. Kit Components

1. (5) packs containing 2 cassettes per pack. One cassette with a test strip impregnated with anti-IgG<sub>1</sub>, anti-IgG<sub>2a</sub> and anti-IgG<sub>2b</sub> specific antibodies; and one cassette with a test strip impregnated with anti-IgG<sub>3</sub>, anti-IgA and anti-IgM specific antibodies.
2. (1) x 25 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  $\mu$ L of ascites fluid to 4 mL of Sample Diluent. Thoroughly mix the sample.
3. Add 150  $\mu$ L 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  $\mu$ L of cell culture supernatant fluid to 0.5 mL of Sample Diluent<sup>1</sup>. Vortex to thoroughly mix the sample.
3. Add 150  $\mu$ L 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.

<sup>1</sup> Dilute the sample 1:10 for supernatants that contain monoclonal antibody at less than 1.0  $\mu$ g/mL by adding 55  $\mu$ L of cell culture supernatant to 500  $\mu$ L of Sample Diluent. Mix thoroughly and continue with Step 3.

## X. 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 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.

## XVII. Additional Products and Services

<b>Additional Products</b>	<b>Code</b>	<b>Size</b>	<b>Price</b>
Aviva Rapid Mouse Monoclonal Isotyping Kit	OKRA00053	1 X 5 pack	<a href="http://www.avivasysbio.com">www.avivasysbio.com</a>
Aviva Rapid Mouse Monoclonal Isotyping Kit	OKRA00052	1 X 10 pack	<a href="http://www.avivasysbio.com">www.avivasysbio.com</a>

Rev.1 (09/2010)