Raybio® Rapid Mouse Ig Isotyping Array

-- One step determination of 8 Mouse Immunoglobulin sub-classes and 2 light chain types in one experiment

Patent Pending Technology

User Manual (Version Dec 2019)

Cat # AAM-ISO-G1

RayBiotech, Inc.

We Provide You With Excellent Protein Array Systems and Service

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I. Overview

Immunoglobulin Detected	Heavy Chains (8): IgA, IgD, IgE, IgM, IgG1, IgG2a, IgG2b, and IgG3; Light Chains (2): κ, and λ
Format	One standard glass slide is spotted with 16 wells of identical Immunoglobulin antibody arrays. Each Ig-specific antibody is arrayed in quadruplicate.
Detection Method	Fluorescence with laser scanner: Cy3 equivalent dye
Sample Volume	1-2 μl
Sample Dilutions	Hybridoma Supernatant: 1:10 – 1:1,000 Purified mouse monoclonal antibody: 10-1000 ng/ml Ascitic fluids/Serum/Plasma: 1:1,000 – 1:100,000
Reproducibility	CV <10%
Assay duration	1 hr

Mouse Ig Array Map

POS1	POS2
IgA	**IgD**
IgE	**IgM**
IgG1	**IgG2a**
IgG2b	**IgG3**
λ	**κ**

TABLE OF CONTENTS

Introduction

Immunoglobulins are the key elements of the vertebrate humoral immune response against pathogen invasion and infection. The polypeptide chains of immunoglobulins are composed of two identical heavy (H) chains and two identical light (L) chains linked together by inter-chain disulfide bonds. While the amino terminal portions that exhibit highly variable amino acid composition are involved in antigen binding, the C terminal constant regions are involved in complement binding, placental and intestinal passage, and binding to cell membranes. Based upon the variation of the constant region of the heavy chain, eight immunoglobulin heavy chain isotypes are found in mice: IgA, IgD, IgE, IgM, and IgG (with subclasses IgG1, IgG2a, IgG2b, and IgG3).

Identification of class and subclass of immunoglobulins is essential for determination of immunochemical and functional properties. Detection of specific Ig isotype is a powerful tool in the study of immunoglobulin-deficiency disorders, allergies, autoimmune diseases, malignancies, GI disorders or repeated bacterial infections. Meanwhile, the growth and widespread use of mouse monoclonal antibody technology have created a need for a fast, accurate, and simple means of determining immunoglobulin class and sub-class. Identification is essential since chemical and biological properties of the various classes are unique. They differ in their solubility and electrophoretic properties, in their susceptibility to cleavage enzymes, and in their reactivity with protein A. Determining the class and subclass of a monoclonal antibody is thus useful in planning the best immunoglobulin purification method. For example, mouse IgA and IgM are best purified by size (i.e., gel exclusion) or using immunoaffinity separation columns. Mouse IgG2a and IgG2b are purified with immobilized Protein A at pH 7-8, while Mouse IgG1 binds best to Protein A at pH 8-9. Immunoglobulin that contains kappa light chains can be purified using immobilized Protein L.

The RayBio® Rapid Mouse Ig Isotyping Array uses sandwich-ELISA based technology for determination of the eight mouse immunoglobulin sub-classes (IgG1, IgG2a, IgG2b, IgG3, IgA, IgD, IgE, and IgM) and the two light chain types. Briefly, the 8 mouse immunoglobulin subclass and 2 light chain specific

antibodies are arrayed in quadruplicate (together with two positive controls) with 16 identical sub-arrays in one standard glass slide. Sixteen samples can be assayed in one slide simultaneously through a sandwich ELISA procedure. While the traditional sandwich-based assay is time consuming and contains multiple steps such as blocking, sample incubation, addition of detection antibody, and signal generation through fluorescence or chemiluminescence detection methods, our one-step mouse Ig isotyping kit uses an innovative one-step strategy which greatly simplifies the whole procedure while retaining similar detection specificity and sensitivity. In this system, samples are first mixed with the Cy3 equivalent dye-labeled detection antibody, and then applied to the array. After washing away the non-specific signals, the slides can then be visualized with a laser scanner. Results can be evaluated qualitatively by visual inspection or semi-quantitatively through data extraction.

The kit provides a highly sensitive approach (within nanogram range) for simultaneous detection of 8 immunoglobulin subclasses and the 2 light chain types in cell culture supernatants or from purified mouse monoclonal antibodies. Because mouse serum, plasma, and ascites fluids contain all the eight Ig isotypes, this assay may be used for disease-associated isotype profiling of these sample types. The abundance of each isotype in each sample can be determined semi-quantitatively. The experimental procedure is simple and can be performed in any laboratory.

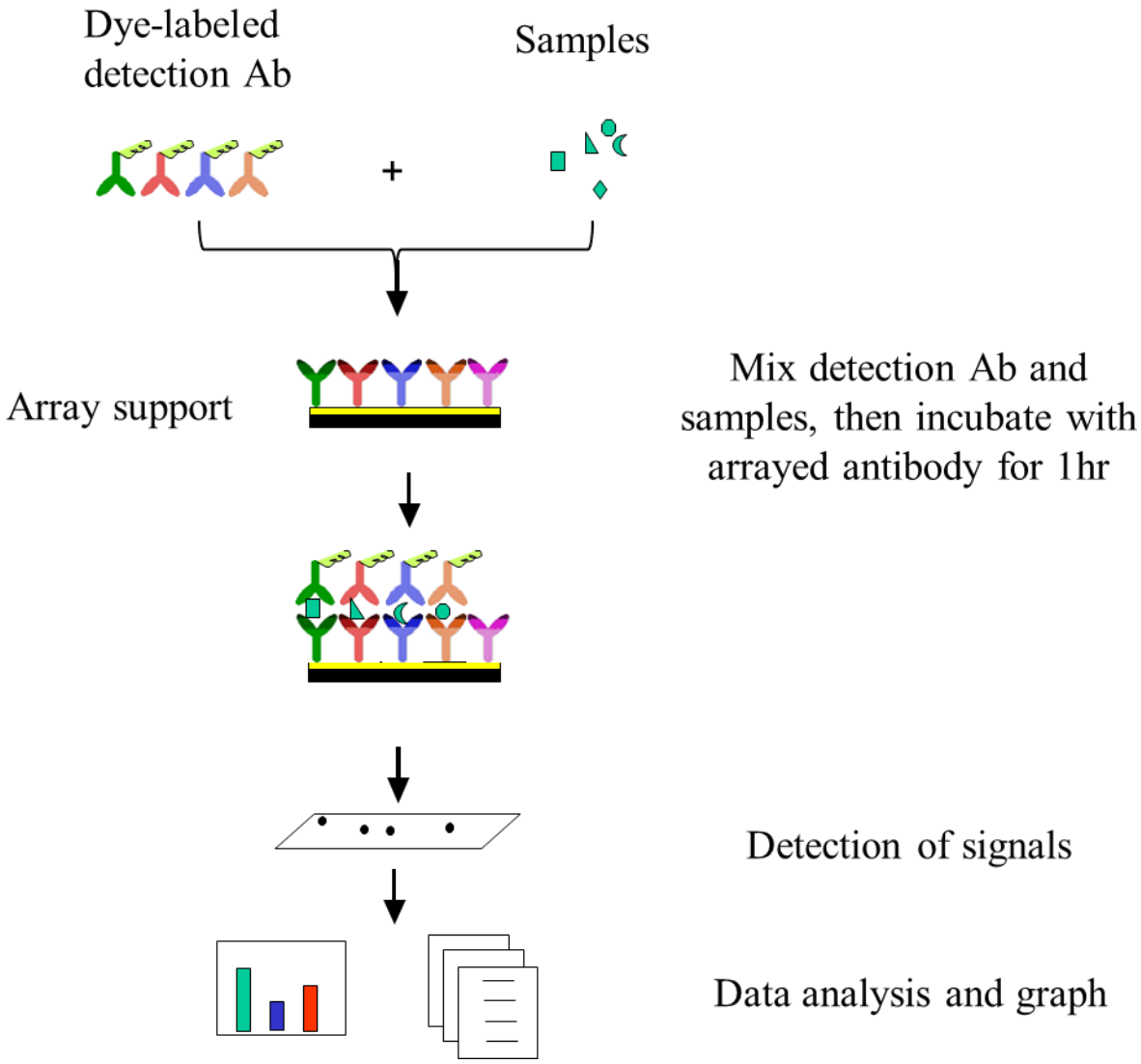
Research Applications

- Antibody-producing hybridoma screening and selection
- Mouse monoclonal antibody heavy and light chain identification
- Selection and isolation of immunoglobulin isotype switch variants
- Mouse model immune disease research (autoimmune disease, allergies, Ig deficiency disorders, malignancies, GI disorders or repeated bacterial infections etc.)

Kit Features

- One step mouse monoclonal antibody isotyping
- Requires only 1-2 μl sample
- Entire experiment can be done within 1 hour
- Sandwich based technology allows for high specificity and sensitivity
- Low system CV with high reproducibility
- High throughput sample processing
- Qualitative visual inspection or semi-quantitative result
- Processed slides can be stored for years without signal decay

How it works



I.

II. Materials Provided

Upon receipt, all components of the One-Step Mouse Ig Isotyping kit should be stored at -20^{0}C. At -20^{0}C the kit will retain complete activity for up to 6 months.

Components

Item	Description	1-Slide kit	2-Slide kit
1	Mouse Ig Isotyping Glass Slide	1	2
2	Sample Diluent	1	2
3	20X Wash Buffer I	1	1
4	Detection Antibody Cocktail	1	2
5	Slide Washer/Dryer	1	1
6	96-well Plate	1	1
7	Manual	1	1

Additional Materials Required

- Orbital shaker
- Laser scanner for fluorescence detection
- Distilled water
- Microcentrifuge

III. General Considerations

A. Sample Preparation

- **Sample types:** This is an Ig isotyping assay for hybridoma supernatant and other purified antibodies. Since serum, plasma, and ascitic fluid contain all eight Ig isotypes, this kit can be used for monitoring the relative Ig isotype abundance through semi-quantitative data analysis.
- **Sample dilution**:
    a) Hybridoma supernatant: 1:10 – 1:1,000
    b) Purified mouse monoclonal antibody: 10 – 1000 ng/ml
    c) Serum, plasma, and ascitic fluids: 1:1,000 – 1:100,000

B. Handling glass slides

- Do not touch the surface of the slides as the microarray slides are very sensitive. Hold the slides by the edges only.
- Handle all buffers and slides with latex free gloves.
- Handle glass slide in clean environment.
- Because there is no barcode on the slide, transcribe the slide serial number from the slide bag to the back of the slide with a permanent marker before discarding the slide bag. Once the slide is disassembled, there might not be enough information to distinguish one slide from another.

C. Incubation

- Completely cover array area with sample or buffer during incubation.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rotation.
- Incubation can be done at room temperature for 1 hr or 30 min at 37^0C.

IV. Protocol

- **Completely air dry the glass slide**

1. Take out the glass slide from the box, and let it equilibrate to room temperature inside the sealed plastic bag for 20-30 minutes. Remove slide from the plastic bag; peel off the cover film, and let it air dry at room temperature for another 1-2 hours.

Note: Incomplete drying of slides before use may cause the formation of "comet tails".

- **Sample Incubation**

2. Add 1.7 ml Sample Diluent into the detection antibody cocktail tube. Spin briefly.

3. Based upon the sample numbers, aliquot 98 µl of the detection antibody to corresponding number of wells in the 96-well plate.

4. Add 2 µl of each sample to each well, mix well through pipetting.

   Note: This dilution is optimized for hybridoma supernatant. For purified mouse monoclonal antibody, dilute the samples with Sample Diluent to 10 µg/ml prior to the kit. For serum/plasma/ascites, add 1ul sample to 99ul Sample Diluent to make a 100x sample dilution first, then use 2 µl for sample testing.

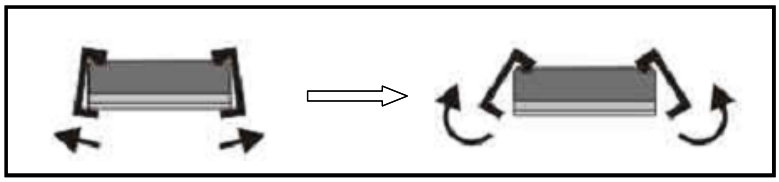
5. Transfer 100 µl of mixed samples to the appropriative wells on the glass slide. Incubate at room temperature for 1 hour (or 30 min in 37^0C).

6. Wash: Decant the samples from each well, and wash 5 times with 1x Wash Buffer I at room temperature. Rinse briefly and then completely remove wash buffer in each wash step. Dilute 20x Wash Buffer I with distilled water.

- **Fluorescence Detection**

7. Disassemble the device by pushing clips outward from the slide side. Carefully remove the slide from the gasket.

(Be careful not to touch the surface of the array side)



(*Optional*): Place the slide in the slide Washer/Dryer, add enough 1x Wash Buffer I (about 30 ml) to cover the whole slide, and then gently shake at room temperature for 15 minutes. Decant Wash Buffer I.

8. Rinse briefly with distilled water, and completely remove water droplets through gentle suction with a pipette. Do not touch the array, only the sides.

9. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength such as Axon GenePix. List of specifications and compatible scanners can be found in Section XI.

- **Data Analysis**

10. Results can be evaluated qualitatively by visual inspection or semi-quantitatively through data extraction with most microarray analysis software (GenePix, ScanArray Express, ArrayVision, or MicroVigene). Our array specific Raybio® Rapid Mouse Ig Isotyping Analyzer software is available for one-step data computation. It outputs digital values as well as isotype names.

V. Specificity & Accuracy

The mouse isotype specific capture antibodies in the kit have been tested to react only with their own isotype and do not react with other mouse Ig isotypes up to 1 μg/ml. The accuracy of the kit is further confirmed by isotype predetermined mouse monoclonal antibody controls and third-party ELISA determined hybridoma supernatant samples.

VI. Assay Sensitivity

The detection sensitivity for each mouse Ig heavy or light chain has been determined with purified mouse immunoglobulin antigens to be in the nano-gram range (see following).

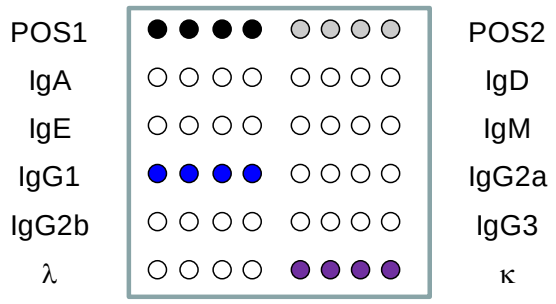
Ig Isotype	Sensitivity (ng/ml)
IgA	1
IgD	ND
IgE	0.4
IgM	4
IgG1	0.1
IgG2a	0.1
IgG2b	0.1
IgG3	1
λ	0.1
κ	0.1

ND: not determined

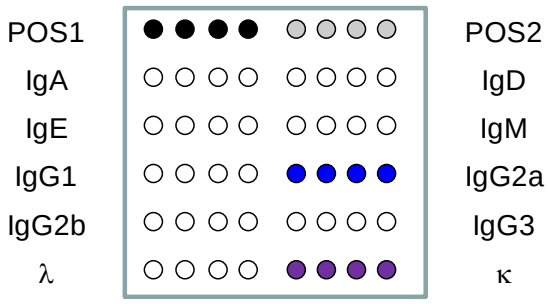
VII. Typical Results

In a typical set of results for hybridoma supernatants or purified monoclonal antibodies, except for the positive controls, only one of the eight heavy chains and one of the light chains will show positive signals in each array (see following examples). For mouse serum, plasma, or ascitic fluids, since it contains all the eight isotypes, the most abundant isotypes (IgG1, IgG2a, IgG2b, IgG3, and IgM) will generally have much stronger signals than the low abundant group (IgA, IgD, and IgE). In general, the light chain **κ** is generally more dominant than **λ** chain.

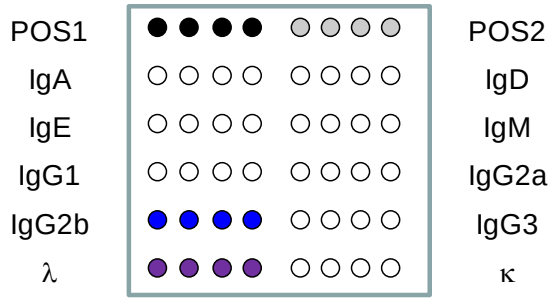
IgG1κ



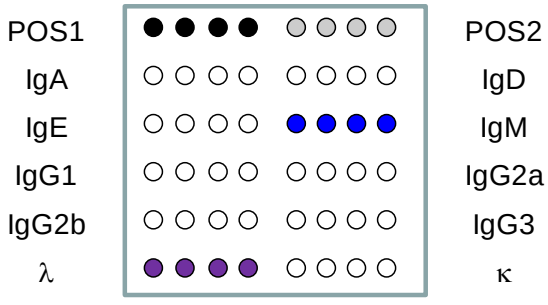
IgG2aκ



IgG2bλ



IgMλ



VIII. Raybio® Rapid Mouse Ig Isotyping Analyzer

Raybio® Rapid Mouse Ig Isotyping Analyzer is an Excel-based program specifically designed for this product. It facilitates the semi-quantitative data analysis as well as output the final sample isotypes. With a simple copy and paste process, the sample Ig isotype is determined.

Semi-quantitative Data Output

Sample	*S1-1*	*S1-2*	*S1-3*	*S1-4*	*S1-5*	*S1-6*	*S1-7*	*S1-8*	*S1-9*	*S1-10*
POS1	12769	13215	11164	10086	11101	11296	11380	11394	11764	12135
POS2	601	627	553	521	547	513	528	585	566	570
IgA	250	187	292	4218	215	230	209	264	238	200
IgD	277	182	292	223	34293	288	277	279	314	300
IgE	210	206	21199	219	2236	205	270	255	244	270
IgM	242	1223	265	218	271	31248	247	221	258	263
IgG1	245	2332	538	308	1763	496	45097	319	311	331
IgG2a	310	355	377	379	976	558	471	543	502	462
IgG2b	256	794	336	512	539	346	294	52420	236	2776
IgG3	225	191	285	268	200	184	253	325	8665	338
Lambda	289	569	273	258	24536	262	275	278	307	6290
Kappa	308	311	59011	3397	542	28882	32306	28953	13222	184

Direct Sample Ig Isotype Output

Sample	**S1-1**	**S1-2**	**S1-3**	**S1-4**	**S1-5**	**S1-6**	**S1-7**	**S1-8**	**S1-9**	**S1-10**
H Chain	**-**	**?**	**IgE**	**IgA**	**IgD**	**IgM**	**IgG1**	**IgG2b**	**IgG3**	**IgG2b**
L Chain	**-**	**?**	**Kappa**	**Kappa**	**Lambda**	**Kappa**	**Kappa**	**Kappa**	**Kappa**	**Lambda**

- **Undetected**
? **Undecided**

IX. Troubleshooting guide

Problem	Cause	Recommendation
Weak Signal	Inadequate detection	Increase laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Short incubation time	Ensure sufficient incubation time or increase sample incubation step to overnight
	Too low antibody concentration in sample	Add more sample
	Improper storage of kit	Store kit as suggested temperature. Don't freeze/thaw the slide.
Uneven signal	Bubble formed during incubation	Avoid bubble formation during incubation
	Arrays are not completed covered by reagent	Completely cover arrays with solution
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
	Comet tail formation	Air dry the slide for at least 1 hour before usage
Multiple heavy chains are detected	Impure sample	Use serum/plasma or ascites free samples; Use fresh culture supernatant or purified antibody
	Hybrodoma sample contains two or more cell lines (polyclonal antibodies)	Reclone hybridoma cells by limited dilution
	Sample too concentrated	Increase dilution of supernatant samples. For purified antibodies, the final concentration should be lower than 1 ug/ml
	Myeloma line used in hybridoma production is secreting antibody	Increase sample dilution
High background	Overexposure	Decrease the laser power
	Dark spots	Completely remove wash buffer after each wash step.
	Insufficient wash	Increase wash time and use more wash buffer
	Dust	Work in clean environment
	Slide is allowed to dry out	Don't dry out slides during experiment.

X. Experiment Record Form

Date: _____

File Name: _____

Laser Power: _____

PMT: _____

Well No.	Sample Name	H Chain	L Chain
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			

XI. Laser Scanners for Glass Slide Arrays

Specifications

- Standard Glass Slide: 1" x 3" (25 mm x 75 mm) microscope slides
- Thickness 1 mm
- Light and Detector Orientation: Facing array
- Scanned Area 22 mm x 73 mm
- Focus Auto focus or adjustable (+/- 200 μm)
- Excitation Cy3 (Green) Channel 532 nm
- Resolution 10 μm
- Dynamic Range >3 orders of magnitude
- Detection Output 16-bit TIFF

Recommended Scanners

- GenePix® 4000A (Molecular Devices Corporation)
- GenePix® 4000B (Molecular Devices Corporation)
- GenePix® 4100A (Molecular Devices Corporation)
- GenePix® Professional 4200A (Molecular Devices Corporation)
- ScanArray® Lite (PerkinElmer, Inc.)
- ScanArray® Express (PerkinElmer, Inc.)
- ScanArray® Express HT (PerkinElmer, Inc.)
- LS Series Laser Scanner (Tecan Group AG)
- AlphaScan Microarray Scanner (Alpha Innotech)
- The DNAscope LM (Biomedical Photometrics Inc.)
- The DNAscope IV & V (Biomedical Photometrics Inc.)
- Open Frame DNAscope (Biomedical Photometrics Inc.)
- Revolution 4200 Microarray Scanner (VIDAR Systems Co)
- aQuire 110V (Genetix)
- aQuire 240V (Genetix)
- VersArray ChipReader 5um System (Bio-Rad)
- VersArray ChipReader 3um System (Bio-Rad)
- InnoScan 710 Microarray Scanner (Innopsys)
- InnoScan 900 Microarray Scanner (Innopsys)

Compatible Scanners

- NovaRay Detection Platform (Alpha Innotech)
- arrayWoRx (Applied Precision, LLC)
- GSD-501 System Calibration Kit (Invitrogen)

Please note that this is not an exhaustive list. In general, most gene microarray scanners should be compatible as long as they have a Cy3 (green) channel, pixel resolution of 10μm and able to scan a standard histology slide.

This product is for research use only.

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