Raybio® Rapid Rat Ig Isotyping Array

-- One step determination of 6 Rat Immunoglobulin sub-classes and 2 light chain types in one experiment

Patent Pending Technology

User Manual (Version Sept 2014)

Cat # AAR-ISO-G1

RayBiotech, Inc.

We Provide You With Excellent Protein Array Systems and Service

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I. Overview

Immunoglobulin Detected	Heavy Chains (6): IgA, IgM, IgG1, IgG2a, IgG2b, IgG2c Light Chains (2): Kappa (κ), Lambda (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 rat monoclonal antibody: 10-1000 ng/ml Ascitic fluids/Serum/Plasma: 1:10,000 – 1:1,000,000
Reproducibility	CV <10%
Assay duration	1 hr

Rat Ig Array Map

POS1	POS2
IgA	**Ig**
IgG1	**IgG2a**
IgG2b	**IgG2c**
λ	**κ**

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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, six immunoglobulin heavy chain isotypes are found in rats: IgA, IgM, IgG1, IgG2a, IgG2b, and IgG2c.

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 rat 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, rat IgA and IgM are best purified by size (i.e., gel exclusion) or using immunoaffinity separation columns. Rat IgG2a and IgG2b are purified with immobilized Protein A at pH 7-8, while rat 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 Rat Ig Isotyping Array uses sandwich-ELISA based technology for determination of the six rat immunoglobulin sub-classes (IgA, IgM, IgG1, IgG2a, IgG2b, and IgG2c) and the two light chain types. Briefly, the 6 rat 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 rat 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 6 immunoglobulin subclasses and the 2 light chain types in cell culture supernatants or from purified rat monoclonal antibodies. Because rat serum, plasma, and ascites fluids contain all the six 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. For researchers who want to quantify each Ig isotype in the rat serum or plasma, a quantitative version of this kit is available (Cat# QAR-ISO-1).

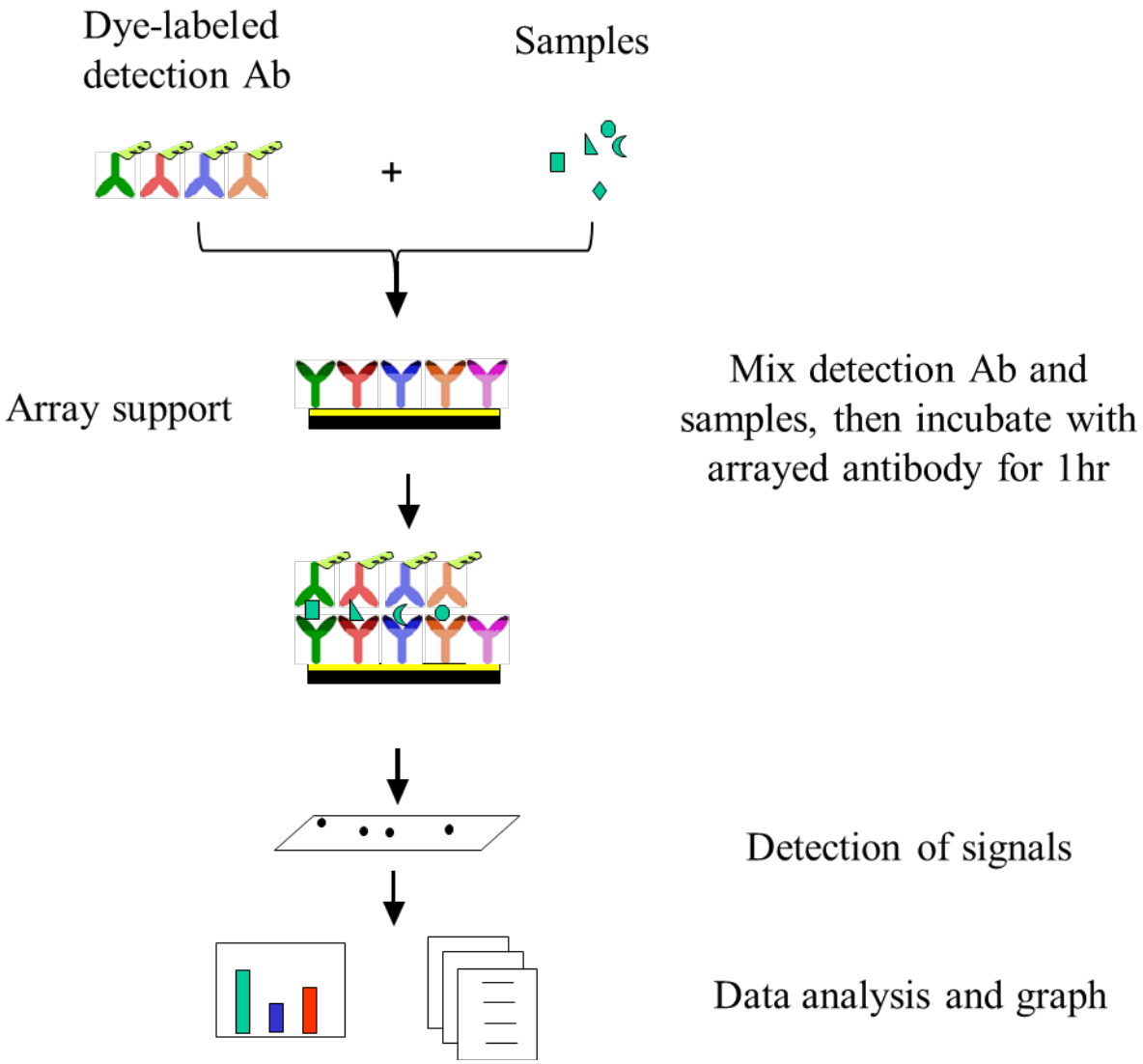
Research Applications

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

Kit Features

- One step rat 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
- Processed slides can be stored for years without signal decay
- Qualitative visual inspection or semi-quantitative result; A quantitative version of this kit is available with the Cat# QAR-ISO-1.

How it works


Dye-labeled detection Ab
Samples
+
Array support
Mix detection Ab and samples, then incubate with arrayed antibody for 1hr
Detection of signals
Data analysis and graph

I. Materials Provided

Upon receipt, all components of the One-Step Rat Ig Isotyping kit should be stored at -20^{0}C. At -20^{0}C the kit will retain complete activity for up to 12 months.

Components

Item	Description	1-Slide kit	2-Slide kit
1	Rat Ig Isotyping Glass Chip	1	2
2	Sample Diluent	1	1
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

II. 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 six 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 rat monoclonal antibody: 10 – 1000 ng/ml
    c) Serum, plasma, and ascitic fluids: 1:10,000 – 1:1,000,000

B. Handling glass chips

- 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 chip 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.

III. Protocol

- **Completely air dry the glass chip**

1. Take out the glass chip 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 100 µl of the detection antibody to corresponding number of wells in the 96-well plate.

4. Add 1 µl of each sample to each well, mix well through pipetting.

   Note: This dilution is optimized for hybridoma supernatant and purified rat monoclonal antibody. For serum/plasma/ascites, add 1ul sample to 999ul Sample Diluent to make a 1000x diluted sample first, then use 1 µl for sample testing.

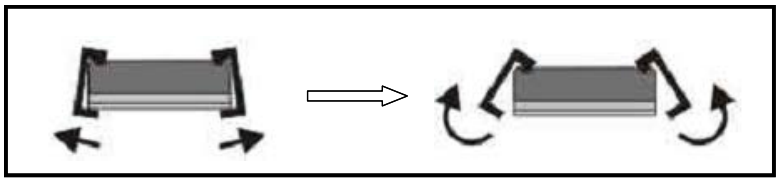
5. Transfer 100 µl of mixed samples to the appropriative wells on the glass slide. Incubate at room temperature for 1 hour (or 30 min in 37^0C).

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 Rat Ig Isotyping Analyzer software is available for one-step data computation. It outputs digital values as well as isotype names.

IV. Specificity & Accuracy

The rat isotype specific capture antibodies in the kit have been tested to react only with their own isotype and do not react with other rat Ig isotypes up to 1 μg/ml. The accuracy of the kit is further confirmed by isotype predetermined rat monoclonal antibody controls.

	IgA kappa	IgM kappa	IgG1 lambda	IgG2a lambda	IgG2b kappa	IgG2c kappa
IgA	16987	228	200	257	205	245
IgM	378	2476	228	450	245	408
IgG1	410	509	5322	505	253	497
IgG2a	376	505	317	4388	338	513
IgG2b	416	494	385	496	4206	444
IgG2c	449	484	380	660	318	13472
Lambda	300	332	16088	9822	241	287
Kappa	10353	4115	378	589	8283	3753

V. Assay Sensitivity

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

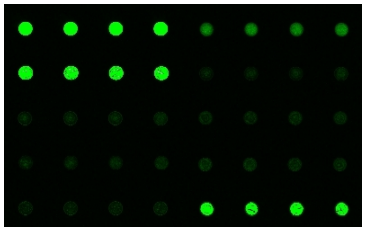
Ig Isotype	Sensitivity (ng/ml)
IgA	0.2
IgM	10
IgG1	0.4
IgG2a	0.1
IgG2b	0.4
IgG2c	0.1
λ	0.1
κ	0.1

VI. Typical Results

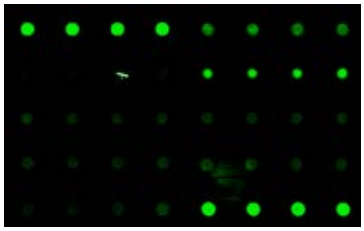
In a typical set of results for hybridoma supernatants or purified monoclonal antibodies, except for the positive controls, only one of the six heavy chains and one of the light chains will show positive signals in each array (see following examples). For rat serum, plasma, or ascitic fluids, since it contains all the six isotypes, the most abundant isotypes (IgG1, IgG2a, IgG2b, and IgG2c) will generally have much stronger signals than the low abundant group (IgA and IgM). In general, the light chain **κ** is generally more dominant than **λ** chain.

Representative Images

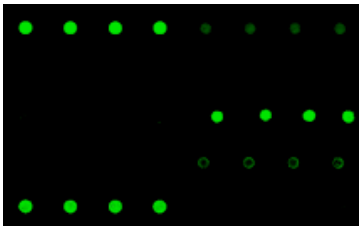
IgA κ



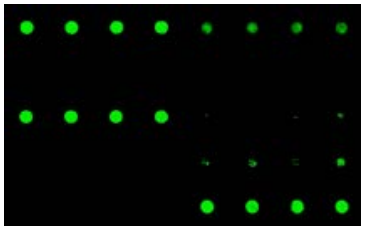
IgM κ



IgG2a λ

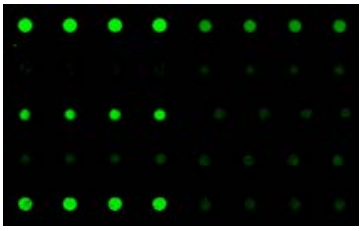


IgG1 κ

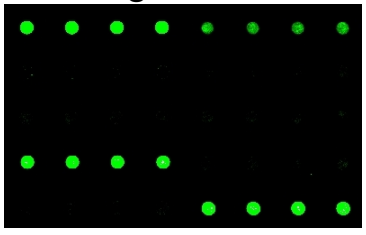


POS1	POS2
IgA	IgM
IgG1	IgG2a
IgG2b	IgG2c
Lambda	Kappa

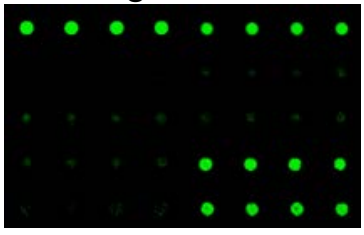
IgG1 λ



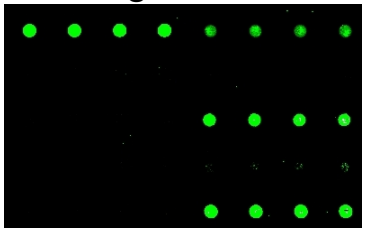
IgG2b κ



IgG2c κ



IgG2a κ



VII. Raybio® Rapid Rat Ig Isotyping Analyzer

Raybio® Rapid Rat 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	31693	29799	31449	30885	32055	30998	30448	30139	30518	32246
POS2	2739	2925	2763	2818	2703	2807	2861	2891	2854	2684
IgA	26066	782	336	396	315	369	320	326	218	313
IgM	581	11891	641	691	375	766	609	624	423	11339
IgG1	629	1333	6082	777	388	812	54342	663	449	872
IgG2a	577	1338	719	6743	518	886	641	67140	533	460
IgG2b	639	1279	703	762	3382	824	674	626	8403	464
IgG2c	689	1378	788	1015	487	49039	890	910	444	477
Lambda	461	983	29705	15094	369	485	456	440	4909	14753
Kappa	15885	30744	711	905	12701	12803	38119	36107	555	444
POS-Ave	29796	29796	29796	29796	29796	29796	29796	29796	29796	29796

Sample Ig Isotypes

	S1-1	S1-2	S1-3	S1-4	S1-5	S1-6	S1-7	S1-8	S1-9	S1-10
H Chain	IgA	IgM	IgG1	IgG2a	IgG2b	IgG2c	IgG1	IgG2a	IgG2b	IgM
L Chain	Kappa	Kappa	Lambda	Lambda	Kappa	Kappa	Kappa	Kappa	Lambda	Lambda

VIII. 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.

IX. 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			

X. 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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