

# Meso Scale Discovery<sup>®</sup>

## MULTI-SPOT<sup>®</sup> Assay System

### Mouse Isotyping Panel 1 Assay Kit

1-Plate Kit

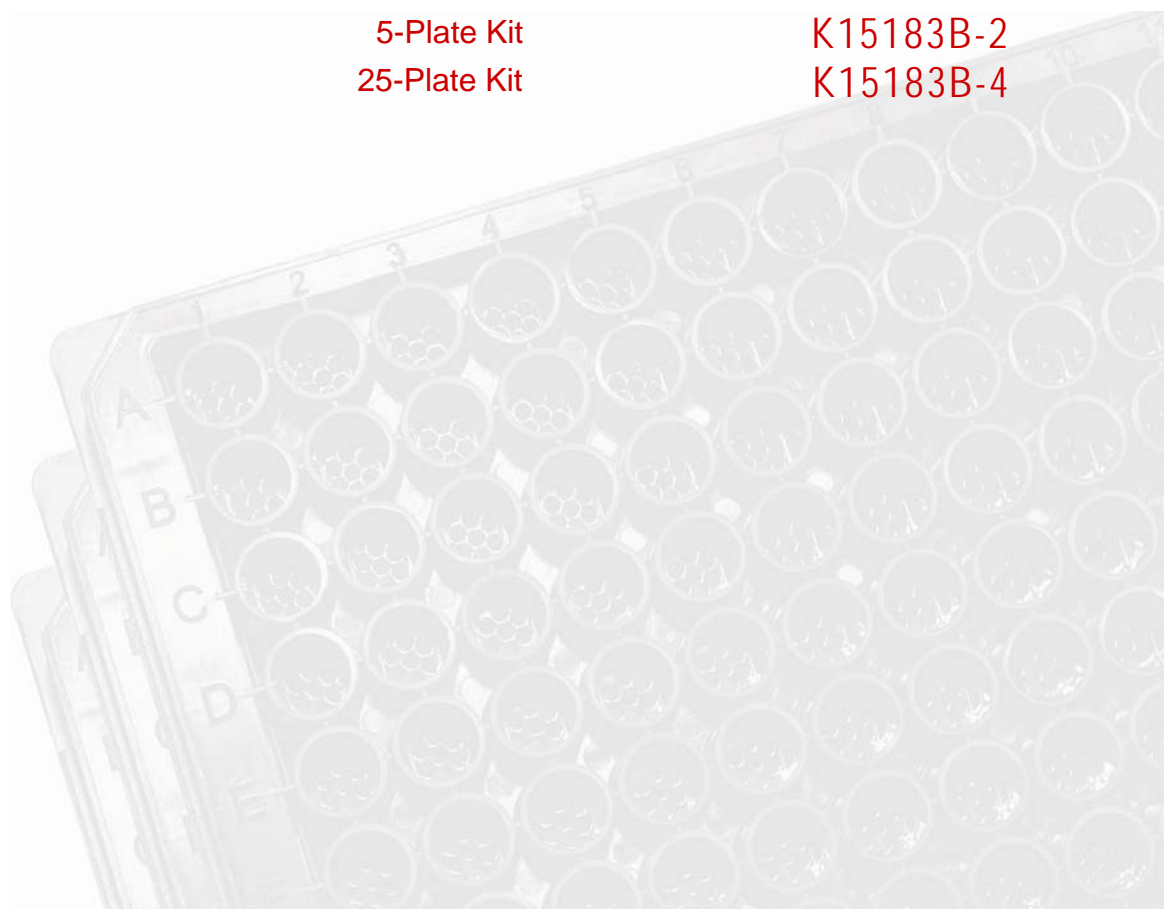
K15183B-1

5-Plate Kit

K15183B-2

25-Plate Kit

K15183B-4



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# MSD MULTI-SPOT Assays

## **Mouse Isotyping Panel 1 Assay Kit**

**IgA, IgG1, IgG2a, IgG2b, IgG3, IgM**

*This package insert must be read in its entirety before using this product.*

**FOR RESEARCH USE ONLY.**

**NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.**

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## Ordering Information

Ordering information

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# Introduction

## introduction

**Immunoglobulins (Ig)** are produced by plasma cells and lymphocytes and are found in serum, urine, spinal fluids, spleen and lymph nodes. Immunoglobulins, also known as antibodies, play a critical role in immune response. They attach to foreign antigens such as bacteria, viruses, fungus and cancer cells and participate in their destruction.

Five primary Ig isotypes have been identified in placental mammals (IgA, IgD, IgE, IgG and IgM) based on the differences of the Fc fragments of their heavy chains. IgG is the most abundant immunoglobulin in serum and is further subclassed into 4 isotypes (IgG1, IgG2a, IgG2b and IgG3). Identification of class and subclass of Ig molecules is essential for determination of immunochemical and functional properties.

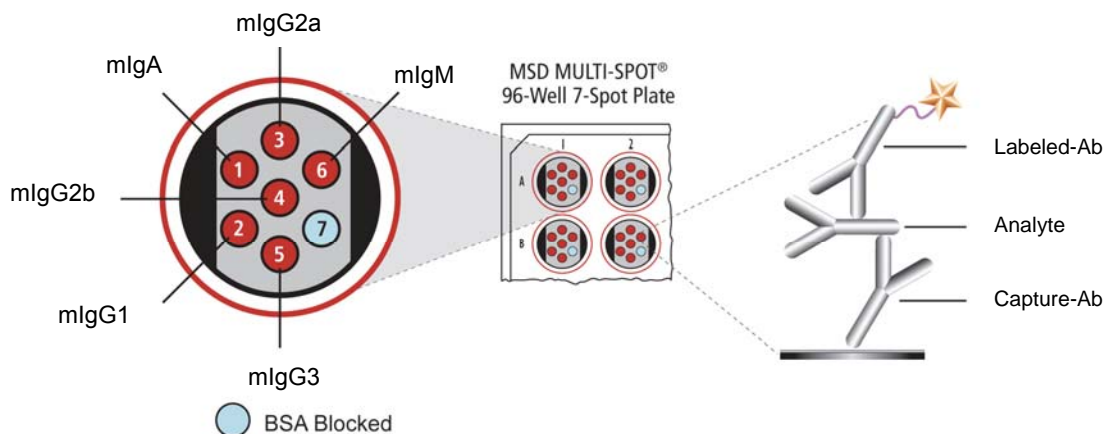
Detection of specific Ig isotype is a powerful tool in the study of immunoglobulin- deficiency disorders, allergies, autoimmune diseases, malignancies, GI disorders or repeated bacterial infections.

**Mouse Isotyping Panel 1 Assay Kit (IgA, IgG1, IgG2a, IgG2b, IgG3, IgM)** enables easy, rapid and simultaneous determination of multiple mouse immunoglobulin classes and subclasses in one well.

# Principle of the Assay

principle of the assay

MSD<sup>®</sup> assays provide a rapid and convenient method for measuring the levels of protein targets within a single small-volume sample. In a multiplex assay, an array of capture antibodies against different targets is patterned on distinct spots in the same well. The Mouse Isotyping Panel 1 Assay detects IgA, IgG1, IgG2a, IgG2b, IgG3 and IgM in a sandwich immunoassay format (Figure 1). MSD provides a plate that has been pre-coated with capture antibody on spatially distinct spots – antibody for IgA, IgG1, IgG2a, IgG2b, IgG3 and IgM. The user adds the sample and a solution containing the labeled detection antibody— anti- IgA, anti- IgG1, anti-IgG2a, anti-IgG2b, anti-IgG3 and anti-IgM labeled with an electrochemiluminescent compound, MSD SULFO-TAG<sup>™</sup> label—over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the labeled detection antibodies by bound analytes completes the sandwich. The user adds an MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD SECTOR<sup>®</sup> instrument for analysis. Inside the SECTOR instrument, a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. The instrument measures intensity of emitted light to afford a quantitative measure of IgA, IgG1, IgG2a, IgG2b, IgG3 and IgM present in the sample.



**Figure 1.** Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

# Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit		
		K15035C-1	K15035C-2	K15035C-4
MULTI-SPOT 96-well 7 Spot Mouse Isotyping Panel 1 Plate N75183A-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG™ Anti-mIgA Antibody <sup>1</sup> (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
SULFO-TAG Anti-mIgG1 Antibody <sup>1</sup> (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
SULFO-TAG Anti-mIgG2a Antibody <sup>1</sup> (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
SULFO-TAG Anti-mIgG2b Antibody <sup>1</sup> (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
SULFO-TAG Anti-mIgG3 Antibody <sup>1</sup> (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
SULFO-TAG Anti-mIgM Antibody <sup>1</sup> (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Mouse Isotyping Panel 1 Calibrator Blend (10 µg/mL)	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	25 vials (15 µL ea)
Diluent 100 R50AA-4 (50 mL) R50AA-2 (200 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	1 bottle (200 mL)
Read Buffer T (4X) R92TC-3 (50 mL) R92TC-2 (200 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	2 bottles (200 mL ea)

## Required Materials and Equipment - not supplied

required materials and equipment — not supplied

- Deionized water for diluting concentrated buffers
- 50 mL tubes for reagent preparation
- 15 mL tubes for reagent preparation
- Microcentrifuge tubes for preparing serial dilutions
- Phosphate buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- Appropriate liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL into a 96-well microtiter plate
- Plate washing equipment: automated plate washer or multichannel pipette
- Adhesive plate seals
- Microtiter plate shaker

<sup>1</sup> Some SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

# V Safety

s a f e t y

Safe laboratory practices and personal protective equipment such as gloves, safety glasses, and lab coats should be used at all times during the handling of all kit components. All hazardous samples should be handled and disposed of properly, in accordance with local, state, and federal guidelines.

# VI Reagent Preparation

r e a g e n t   p r e p a r a t i o n

Bring all reagents to room temperature and thaw the Calibrator stock on ice.

## Prepare Calibrator and Control Solutions

MSD recommends the preparation of an 8-point standard curve consisting of at least 2 replicates of each point. Each well requires 25  $\mu\text{L}$  of Calibrator. For the assay, MSD recommends 4-fold serial dilution steps and Diluent 100 alone for the 8<sup>th</sup> point:

Standard	Mouse Isotyping Panel 1 (pg/mL)	Dilution Factor
100X Stock	10000000	
STD-01	100000	100
STD-02	25000	4
STD-03	6250	4
STD-04	1560	4
STD-05	390	4
STD-06	98	4
STD-07	24	4
STD-08	0	n/a

To prepare this 8-point standard curve for up to 4 replicates:

- 1) Prepare the highest Calibrator point (STD-01) by transferring 10  $\mu\text{L}$  of the Mouse Isotyping Panel 1 Calibrator Blend to 990  $\mu\text{L}$  Diluent 100.
- 2) Prepare the next Calibrator by transferring 50  $\mu\text{L}$  of the diluted Calibrator to 150  $\mu\text{L}$  of Diluent 100. Repeat 4-fold serial dilutions 5 additional times to generate 7 Calibrators.
- 3) The recommended 8<sup>th</sup> Standard is Diluent 100 (i.e. zero Calibrator).

### Notes:

- a. Alternatively, Calibrators can be prepared in the sample matrix or diluent of choice to verify acceptable performance in these matrices. In general, the presence of some protein (for example, 1% BSA) in the sample matrix is helpful for preventing loss of analyte by adsorption onto the sides of tubes, pipette tips, and other surfaces. If your sample matrix is serum-free tissue culture media, then the addition of 10% FBS or 1% BSA is recommended.
- b. The standard curve can be modified as necessary to meet specific assay requirements.

## Dilution of Samples

### *Serum and Plasma*

All solid material should be removed by centrifugation. Plasma prepared in heparin tubes commonly displays additional clotting following the thawing of the sample. Remove any additional clotted material by centrifugation. Avoid multiple freeze/thaw cycles for serum and plasma samples. Some analytes in this matrix are extremely sensitive to multiple freeze/thaw cycles and the ability to detect these analytes may decrease following the first round of thawing. Serum and plasma samples may need to be diluted from 1000-fold to 100000-fold depending on the application. A simple PBS based diluent with 1% BSA may be used for dilution. Alternatively, additional Diluent 100 can be purchased for diluting samples (catalog numbers are provided on page 6).

### *Tissue Culture*

Tissue culture supernatant samples may not require any dilution prior to being used in the MSD Mouse Isotyping Panel 1 Assay. Samples from experimental conditions with extremely high levels of mouse Ig may require a dilution.

## Prepare Detection Antibody Solution

The Detection Antibodies are provided as a 50X stock solution. The working Detection Antibody Solution should contain 1X as final concentration of each antibody.

In a 15 mL tube combine (per plate):

- 60 µL of 50X SULFO-TAG Anti-mIgA Antibody
- 60 µL of 50X SULFO-TAG Anti-mIgG1 Antibody
- 60 µL of 50X SULFO-TAG Anti-mIgG2a Antibody
- 60 µL of 50X SULFO-TAG Anti-mIgG2b Antibody
- 60 µL of 50X SULFO-TAG Anti-mIgG3 Antibody
- 60 µL of 50X SULFO-TAG Anti-mIgM Antibody
- 2640 µL of Diluent 100

## Prepare Read Buffer

The Read Buffer should be diluted 2-fold in deionized water to make a final concentration of 2X Read Buffer T. Add 10 mL of 4X Read Buffer T to 10 mL of deionized water for each plate.

## Prepare MSD Plate

This plate has been pre-coated with antibody for the analyte shown in Figure 1. The plate can be used as delivered; no additional preparation (e.g., pre-wetting) is required. The plate has also been exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies.

# VII Assay Protocol

## assay protocol

1. **Addition of Diluent 100:** Dispense 25  $\mu\text{L}$  of Diluent 100 into each well. Seal the plate with an adhesive plate seal and incubate for 30 min with vigorous shaking (300–1000 rpm) at room temperature.
2. **Addition of the Sample or Calibrator:** Dispense 25  $\mu\text{L}$  of sample or Calibrator into separate wells of the MSD plate. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
3. **Wash and Addition of the Detection Antibody Solution:** Wash the plate 3X with PBS-T. Dispense 25  $\mu\text{L}$  of the 1X Detection Antibody Solution into each well of the MSD plate. Seal the plate and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
4. **Wash and Read:** Wash the plate 3X with PBS-T. Add 150  $\mu\text{L}$  of 2X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. Plates may be read immediately after the addition of Read Buffer.

### Notes

*Shaking a 96-well MSD plate typically accelerates capture at the working electrode.*

*Bubbles in the fluid will interfere with reliable reading of plate. Use reverse pipetting techniques to insure bubbles are not created when dispensing the Read Buffer.*

# VIII Analysis of Results

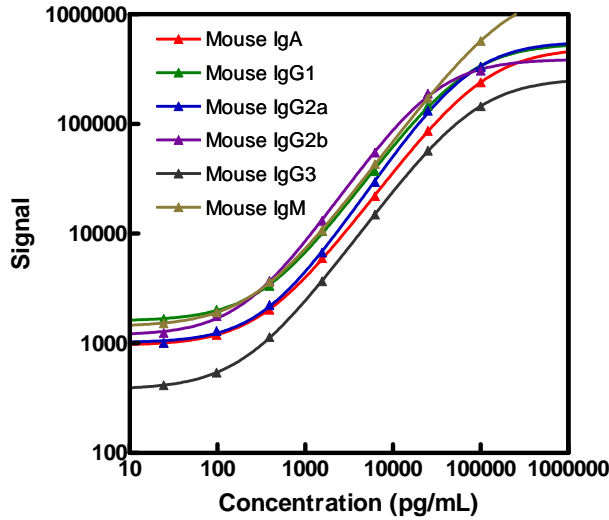
## analysis of results

The Calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have a wide dynamic range (3–4 logs) which allows accurate quantitation in many samples without the need for dilution. The MSD Discovery Workbench<sup>®</sup> analysis software utilizes a 4-parameter logistic model (or sigmoidal dose-response) and includes a  $1/Y^2$  weighting function. The weighting function is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

# IX Typical Standard Curve

typical standard curve

The following standard curve is an example of the dynamic range of the assay. The actual signals may vary and a standard curve should be run for each set of samples and on each plate for the best quantitation of unknown samples.



IgA		
Conc. (pg/mL)	Average Signal	%CV
0	871	4.2
24	1009	6.3
98	1195	6.7
391	2023	7.9
1563	5989	5.6
6250	21962	4.1
25000	86434	6.0
100000	239062	3.0

IgG1		
Conc. (pg/mL)	Average Signal	%CV
0	1475	6.9
24	1670	6.6
98	2022	6.4
391	3329	12.8
1563	10548	11.2
6250	37131	11.6
25000	146374	5.5
100000	334605	6.9

IgG2a		
Conc. (pg/mL)	Average Signal	%CV
0	869	3.2
24	1010	4.4
98	1297	6.9
391	2234	10.9
1563	6719	11.8
6250	29516	8.6
25000	132417	7.2
100000	332372	4.2

IgG2b		
Conc. (pg/mL)	Average Signal	%CV
0	1029	2.4
24	1240	3.0
98	1766	5.3
391	3710	13.5
1563	13084	4.6
6250	54680	5.0
25000	189274	3.7
100000	306598	4.3

IgG3		
Conc. (pg/mL)	Average Signal	%CV
0	348	6.5
24	413	6.4
98	541	2.6
391	1138	8.2
1563	3669	5.2
6250	14973	7.7
25000	56979	4.7
100000	144566	3.6

IgM		
Conc. (pg/mL)	Average Signal	%CV
0	1329	4.1
24	1520	7.3
98	1913	2.7
391	3616	6.1
1563	10660	5.3
6250	43150	3.1
25000	171383	3.3
100000	570171	10.5

# X Sensitivity

sensitivity

The lower limit of detection (LLOD) is the calculated concentration of the signal that is 2.5 standard deviations over the zero calibrator. The value below represents the average LLOD over multiple kit lots.

	IgA	IgG1	IgG2a	IgG2b	IgG3	IgGM
LLOD (pg/mL)	33	45	24	11	34	22

# XI Spike Recovery

spike recovery

Tissue culture media was spiked with Calibrators at multiple values throughout the range of the assay. Each spike was done in  $\geq 3$  replicates.

% Recovery = measured / expected x 100

IgA	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Tissue Culture Media	0	0		
	2000	1554	4.8	78
	20000	16382	7.4	82

IgG1	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Tissue Culture Media	0	0		
	2000	1414	2.5	71
	20000	15560	1.7	78

IgG2a	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Tissue Culture Media	0	0		
	2000	1525	13.6	76
	20000	15881	11.3	79

IgG2b	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Tissue Culture Media	0	0		
	2000	1614	10.5	81
	20000	17373	4.7	87

IgG3	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Tissue Culture Media	0	0		
	2000	1653	4.9	83
	20000	15485	6.9	77

IgM	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Tissue Culture Media	0	0		
	2000	1613	6.1	81
	20000	16188	11.7	81

# XII Linearity

linearity

Tissue culture media was spiked with Calibrators and then diluted with Diluent 100. The concentrations shown below have been corrected for dilution (concentration = measured x dilution factor). Percent recovery is calculated as the measured concentration divided by the concentration of the previous dilution (expected).

% Recovery = (measured x dilution factor) / expected x 100

Sample	Fold Dilution	IgA			IgG1		
		Conc. (pg/mL)	Conc. % CV	% Recovery	Conc. (pg/mL)	Conc. % CV	% Recovery
Tissue Culture Media	1	5558	7.6		6013	3.5	
	2	5543	7.3	100	5416	2.4	90
	4	5431	9.3	98	5520	8.6	102
	8	5457	15.7	100	5543	2.3	100

Sample	Fold Dilution	IgG2a			IgG2b		
		Conc. (pg/mL)	Conc. % CV	% Recovery	Conc. (pg/mL)	Conc. % CV	% Recovery
Tissue Culture Media	1	5846	3.4		5711	7.2	
	2	5679	6.8	97	5481	7.6	96
	4	5428	6.7	96	5497	4.9	100
	8	5331	5.8	98	5659	9.6	103

Sample	Fold Dilution	IgG3			IgM		
		Conc. (pg/mL)	Conc. % CV	% Recovery	Conc. (pg/mL)	Conc. % CV	% Recovery
Tissue Culture Media	1	5658	3.8		4959	4.4	
	2	5402	3.4	95	5532	8.7	112
	4	5840	5.1	108	5618	8.5	102
	8	5331	9.6	91	4930	4.7	88

Pooled serum, EDTA plasma and heparin plasma were diluted with Diluent 100.

Sample	Fold Dilution	IgA			IgG1		
		Conc. (µg/mL)	Conc. % CV	% Recovery	Conc. (µg/mL)	Conc. % CV	% Recovery
Serum	10000	51	17.0		640	1.1	
	20000	51	2.2	101	829	6.9	130
	40000	58	2.4	113	851	6.2	103
	80000	57	4.9	97	851	3.3	100
EDTA Plasma	10000	36	9.9		599	11.8	
	20000	39	5.2	107	713	2.2	119
	40000	41	3.8	106	728	9.2	102
	80000	46	1.2	111	707	9.9	97
Heparin Plasma	10000	79	4.1		778	5.9	
	20000	82	10.7	103	857	10.3	110
	40000	91	3.1	111	978	3.9	114
	80000	94	9.9	104	887	7.2	91

Sample	Fold Dilution	IgG2a			IgG2b		
		Conc. (µg/mL)	Conc. % CV	% Recovery	Conc. (µg/mL)	Conc. % CV	% Recovery
Serum Plasma	10000	350	2.3		180	12.7	
	20000	458	3.6	131	196	9.3	108
	40000	547	6.2	119	207	1.3	106
	80000	584	1.9	107	211	7.3	102
EDTA Plasma	10000	362	7.7		197	7.2	
	20000	476	3.5	132	233	9.7	118
	40000	586	3.7	123	272	3.1	117
	80000	655	6.9	112	281	9.3	103
Heparin Plasma	10000	427	5.9		281	8.9	
	20000	593	4.0	139	347	9.6	124
	40000	801	4.3	135	379	3.0	109
	80000	867	1.9	108	388	13.7	102

Sample	Fold Dilution	IgG3			IgM		
		Conc. (µg/mL)	Conc. % CV	% Recovery	Conc. (µg/mL)	Conc. % CV	% Recovery
Serum Plasma	10000	109	5.6		223	3.0	
	20000	115	3.0	105	209	3.2	94
	40000	121	5.7	105	216	3.2	104
	80000	121	3.0	100	222	5.5	103
EDTA Plasma	10000	107	3.9		164	12.9	
	20000	128	2.0	120	172	10.9	105
	40000	134	3.5	105	173	9.4	101
	80000	139	3.9	103	165	8.4	95
Heparin Plasma	10000	179	4.9		216	10.7	
	20000	186	7.7	104	211	17.8	98
	40000	194	7.2	104	208	12.7	99
	80000	207	4.9	106	203	11.0	97

## XIII Specificity

### specificity

The specificity of the mouse isotyping assays was evaluated by measuring individual spiked immunoglobulin Calibrators at 6.25 ng/mL in each assay.

The table below shows the % cross-reactivity of each assay for each Calibrator.

Assay	Spiked Calibrator % Cross-Reactivity					
	IgA	IgG1	IgG2a	IgG2b	IgG3	IgM
IgA	<b>100</b>	0	0	2.5	0	0.1
IgG1	0	<b>100</b>	0	0.2	0	0
IgG2a	0	0	<b>100</b>	0.4	0.1	1.2
IgG2b	0	0.1	0	<b>100</b>	0	0
IgG3	0	0.1	0	1.5	<b>100</b>	0.1
IgM	0	0	0	0	2.3	<b>100</b>

## Summary Protocol

### MSD 96-well MULTI-ARRAY Mouse Isotyping Panel 1 Assay Kit

MSD provides this summary protocol for your convenience.

Please read the entire detailed protocol prior to performing the MSD Mouse Isotyping Panel 1 Assay.

#### Step 1 : Sample and Reagent Preparation

Bring all reagents to room temperature and thaw the Calibrator stock on ice.

If necessary, samples should be diluted in Diluent 100.

Prepare calibrator solutions and standard curve.

Use the 10 µg/mL Calibrator stock to prepare an 8-point standard curve by diluting in Diluent 100.

**Note:** *The standard curve can be modified as necessary to meet specific assay requirements.*

Prepare Detection Antibody Solution by diluting Detection Antibody to 1X in 3.0 mL of Diluent 100 (per plate).

Prepare 20 mL of 2X Read Buffer T by diluting 4X MSD Read Buffer T with deionized water.

#### Step 2: Add Diluent 100

Dispense 25 µL/well Diluent 100.

Incubate at room temperature with vigorous shaking (300-1000 rpm) for 30 minutes.

#### Step 3: Add Sample or Calibrator

Dispense 25 µL/well Calibrator or sample.

Incubate at room temperature with vigorous shaking (300-1000 rpm) for 2 hours.

#### Step 4: Wash and Add Detection Antibody Solution

Wash plate 3X with PBS-T.

Dispense 25 µL/well 1X Detection Antibody Solution.

Incubate at room temperature with vigorous shaking (300-1000 rpm) for 2 hours.

#### Step 5: Wash and Read Plate

Wash plate 3X with PBS-T.

Dispense 150 µL/well 2X Read Buffer T.

Analyze plate on SECTOR Imager instrument.



