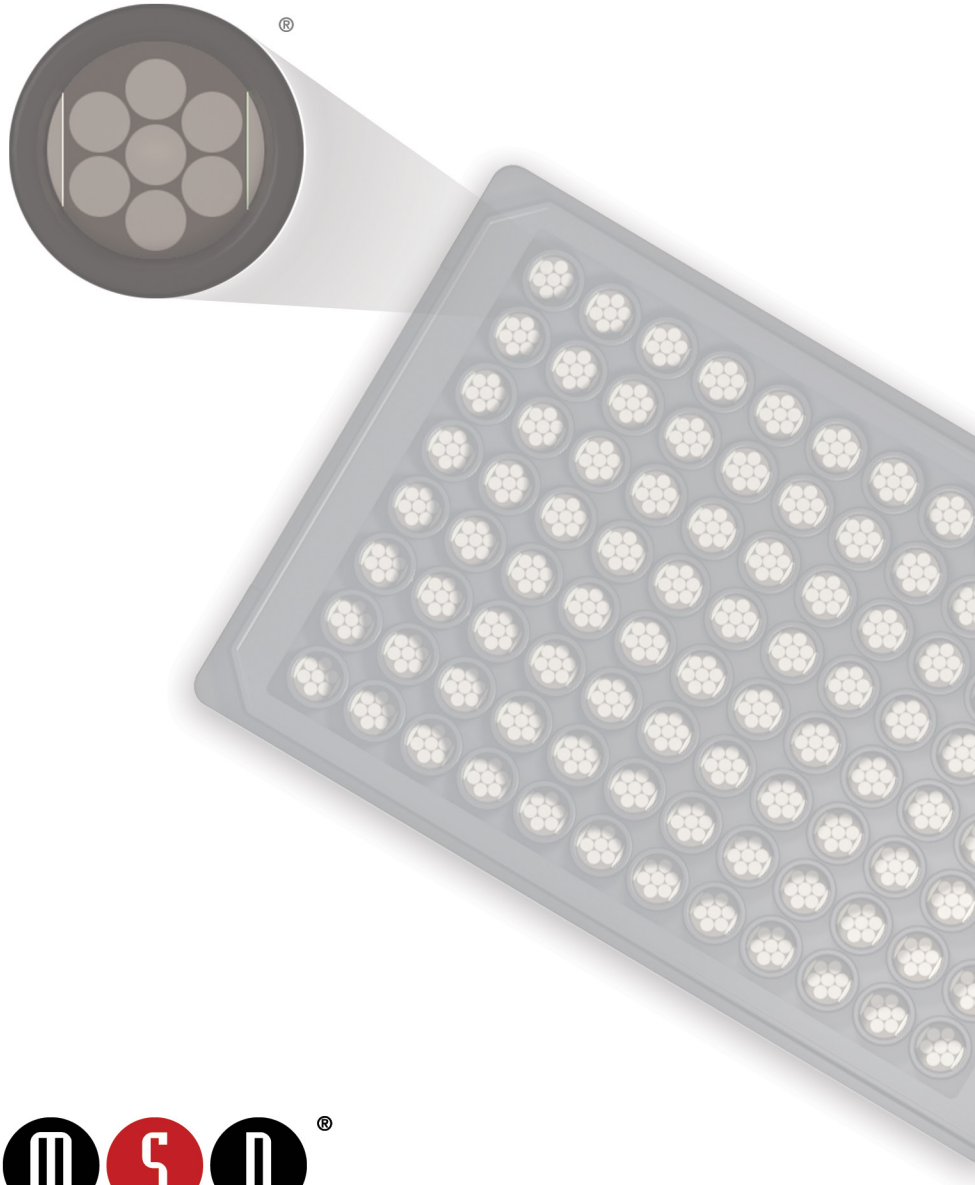
MSD® MULTI-SPOT Assay System

Mouse Isotyping Panel 1 Kit

IgA, IgG1, IgG2a, IgG2b, IgG3, IgM

T-PLEX™



1-Plate Kit	K15183B-1
5-Plate Kit	K15183B-2
25-Plate Kit	K15183B-4

MSD MULTI-SPOT Assays

Mouse Isotyping Panel 1 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 PROCEDURES.

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Table of Contents

Introduction 4
Principle of the Assay 4
Reagents Supplied 5
Required Material and Equipment (not supplied) 6
Safety 7
Reagent Preparation 7
Assay Protocol 9
Analysis of Results 9
Typical Standard Curve 10
Sensitivity 10
Spike Recovery 11
Dilution Linearity 11
Specificity 14
Summary Protocol 14
Plate Diagram 16

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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 the immune response. They attach to foreign antigens such as bacteria, viruses, fungi, 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 subclassified into 4 isotypes (IgG1, IgG2a, IgG2b, and IgG3). Identification of class and subclass of Ig molecules is essential for the determination of their immunochemical and functional properties.

Detection of specific Ig isotypes 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

MSD 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 arranged 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 precoated 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™ label—throughout one or more incubations. 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 MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD® instrument for analysis. Inside the instrument, a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. The instrument measures the intensity of emitted light and provides a quantitative measure of IgA, IgG1, IgG2a, IgG2b, IgG3, or IgM present in the sample.

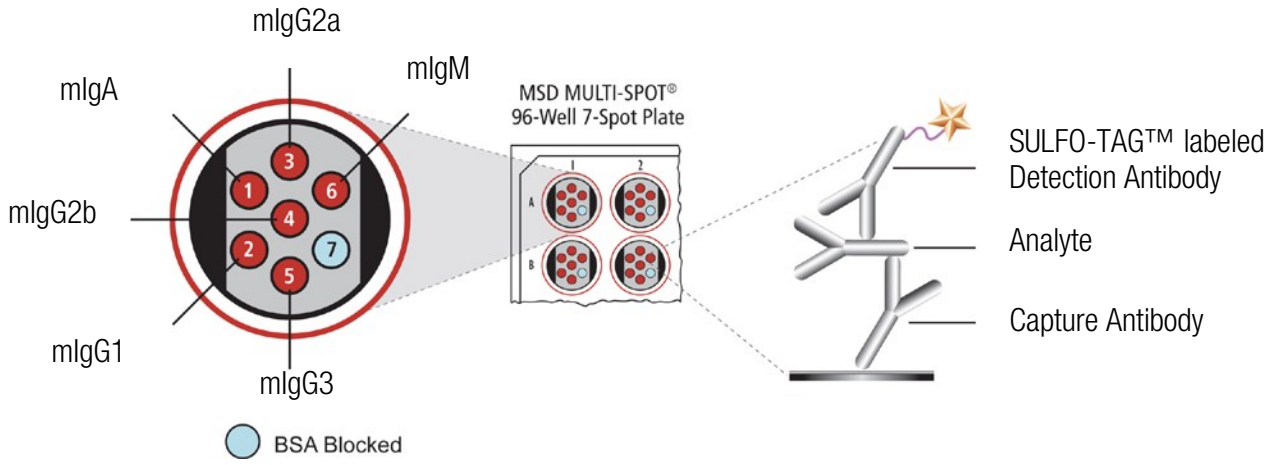


Figure 1. *Spot diagram showing the placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique barcode label on each plate allows complete traceability back to MSD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

Reagents Supplied

Product Description	Storage	Quantity per Kit K15183B-1	K15183B-2	K15183B-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[1] (50X)	2–8 °C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea.)
SULFO-TAG Anti-mIgG1 Antibody[1] (50X)	2–8 °C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea.)
SULFO-TAG Anti-mIgG2a Antibody[1] (50X)	2–8 °C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea.)
SULFO-TAG Anti-mIgG2b Antibody[1] (50X)	2–8 °C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea.)
SULFO-TAG Anti-mIgG3 Antibody[1] (50X)	2–8 °C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea.)
SULFO-TAG Anti-mIgM Antibody[1] (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 Material and Equipment (not supplied)

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

[1] SULFO-TAG conjugated detection antibodies should be stored in the dark.

Safety

Use safe laboratory practices; wear gloves, safety glasses, and lab coats when handling assay components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Additional product-specific safety information is available in the applicable safety data sheets(s) (SDS), which can be obtained from MSD Customer Service or at the www.mesoscale.com® website.

Reagent Preparation

Bring all reagents to room temperature. Thaw the stock calibrator blend 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 µL of calibrator. For the assay, MSD recommends 4-fold serial dilution steps and Diluent 100 alone for the 8th 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

Dash (—) = not applicable

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

1) Prepare the highest calibrator point (STD-01) by transferring 10 µL of the Mouse Isotyping Panel 1 calibrator blend to 990 µL Diluent 100.
2) Prepare the next calibrator by transferring 50 µL of the diluted calibrator to 150 µL of Diluent 100. Repeat 4-fold serial dilutions 5 additional times to generate 7 Calibrators.
3) The recommended 8th Standard is Diluent 100 (i.e. zero calibrator).

Note:

Alternatively, calibrators can be prepared in the sample matrix or diluent of choice to verify the acceptable performance in these matrices. In general, the presence of some protein (for example, 1% BSA) in the sample matrix helps prevent 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.

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

Dilute 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 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 before 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

MSD provides each detection antibody in a 50X stock solution. The working detection antibody solution is 1X.

For 1 plate, combine:

- ❑ 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
- ❑ 2,640 µL of Diluent 100

Note: You may omit detection antibody for any analyte not being measured; add 60 µL of Diluent 100 for each omitted antibody.

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 2X.

For 1 plate, combine:

- ❑ 10 mL of Read Buffer T (4X)
- ❑ 10 mL of deionized water

You may prepare diluted read buffer in advance and store it at room temperature in a tightly sealed container.

Prepare MSD Plate

MSD plates are precoated with capture antibodies (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates can be used as delivered; no additional preparation (e.g., prewetting) is required.

Assay Protocol

Notes

1. **Add Diluent 100:** Add 25 µL of Diluent 100 to 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. **Add Sample or Calibrator:** Add 25 µL of sample or calibrator per well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300-1000 rpm) at room temperature.

   You may prepare detection antibody solution during incubation.

Shaking the plate typically accelerates capture at the working electrode.

3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 25 µL of 1X detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300-1000 rpm) at room temperature.

   You may prepare diluted read buffer during incubation.

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

4. **Wash and Read:** Wash the plate 3 times with 300 µL/well PBS-T. Add 150 µL of 2X Read Buffer T to each well. Analyze the plate on an MSD instrument. No incubation in read buffer is required before reading the plate.

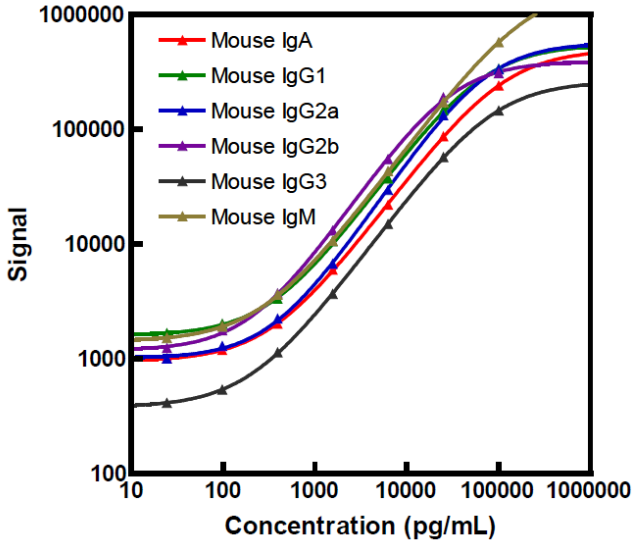
Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods

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 measurement in many samples without the need for dilution. The MSD DISCOVERY WORKBENCH® 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.

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 measurement 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

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	IgM
LLOD (pg/mL)	33	45	24	11	34	22

Spike Recovery

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

% recovery = measured concentration / expected concentration × 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

Dilution 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 concentration × dilution factor). Percent recovery is calculated as the measured concentration divided by the concentration of the previous dilution (expected).

% recovery = (measured concentration × dilution factor) / expected 100

		IgA			IgG1		
Sample	**Fold Dilution**	**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

		IgG2a			IgG2b		
Sample	**Fold Dilution**	**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

		IgG3			IgM		
Sample	**Fold Dilution**	**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.

		IgA			IgG1			IgG2a		
Sample	**Fold Dilution**	**Conc. (µg/mL)**	**Conc % CV**	**% Recovery**	**Conc. (µg/mL)**	**Conc. % CV**	**% Recovery**	**Conc. (µg/mL)**	**Conc. % CV**	**% Recovery**
Serum	10000	51	17.0	—	640	1.1	—	350	2.3	—
	20000	51	2.2	101	829	6.9	130	458	3.6	131
	40000	58	2.4	113	851	6.2	103	547	6.2	119
	80000	57	4.9	97	851	3.3	100	584	1.9	107
EDTA Plasma	10000	36	9.9	—	599	11.8	—	362	7.7	—
	20000	39	5.2	107	713	2.2	119	476	3.5	132
	40000	41	3.8	106	728	9.2	102	586	3.7	123
	80000	46	1.2	111	707	9.9	97	655	6.9	112
Heparin Plasma	10000	79	4.1	—	778	5.9	—	427	5.9	—
	20000	82	10.7	103	857	10.3	110	593	4.0	139
	40000	91	3.1	111	978	3.9	114	801	4.3	135
	80000	94	9.9	104	887	7.2	91	867	1.9	108

		IgG2b			IgG3			IgM		
Sample	**Fold Dilution**	**Conc. (µg/mL)**	**Conc % CV**	**% Recovery**	**Conc. (µg/mL)**	**Conc. % CV**	**% Recovery**	**Conc. (µg/mL)**	**Conc. % CV**	**% Recovery**
Serum	10000	180	12.7	—	109	5.6	—	223	3.0	—
	20000	196	9.3	108	115	3.0	105	209	3.2	94
	40000	207	1.3	106	121	5.7	105	216	3.2	104
	80000	211	7.3	102	121	3.0	100	222	5.5	103
EDTA Plasma	10000	197	7.2	—	107	3.9	—	164	12.9	—
	20000	233	9.7	118	128	2.0	120	172	10.9	105
	40000	272	3.1	117	134	3.5	105	173	9.4	101
	80000	281	9.3	103	139	3.9	103	165	8.4	95
Heparin Plasma	10000	281	8.9	—	179	4.9	—	216	10.7	—
	20000	347	9.6	124	186	7.7	104	211	17.8	98
	40000	379	3.0	109	194	7.2	104	208	12.7	99
	80000	388	13.7	102	207	4.9	106	203	11.0	97

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.

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

Note: IgG2c immunoglobulin does cross-react approximately 2.7% at a concentration greater than 6.25 ng/mL with the IgG2a assay. No cross-reactivity was observed when tested at an IgG2c concentration below 0.391 ng/mL.

Summary Protocol

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

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol before performing the MSD Mouse Isotyping Panel 1 assays.

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature and thaw the calibrator on ice.
- ❑ If necessary, samples should be diluted in Diluent 100.
- ❑ Prepare calibrator solutions and standard curve:

  Note: 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 each 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 1: Add Diluent 100

- ❑ Add 25 µL/well of Diluent 100.
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 30 minutes.

Step 2: Add Sample or Calibrator

- ❑ Add 25 µL/well of calibrator or sample.
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 3: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with 300 µL/well of PBS-T.
- ❑ Add 25 µL/well of 1X detection antibody solution.
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 4: Wash and Read Plate

- ❑ Wash the plate 3 times with 300 µL/well PBS-T.
- ❑ Add 150 µL/well of 2X Read Buffer T.
- ❑ Analyze the plate on an MSD instrument.

Plate Diagram

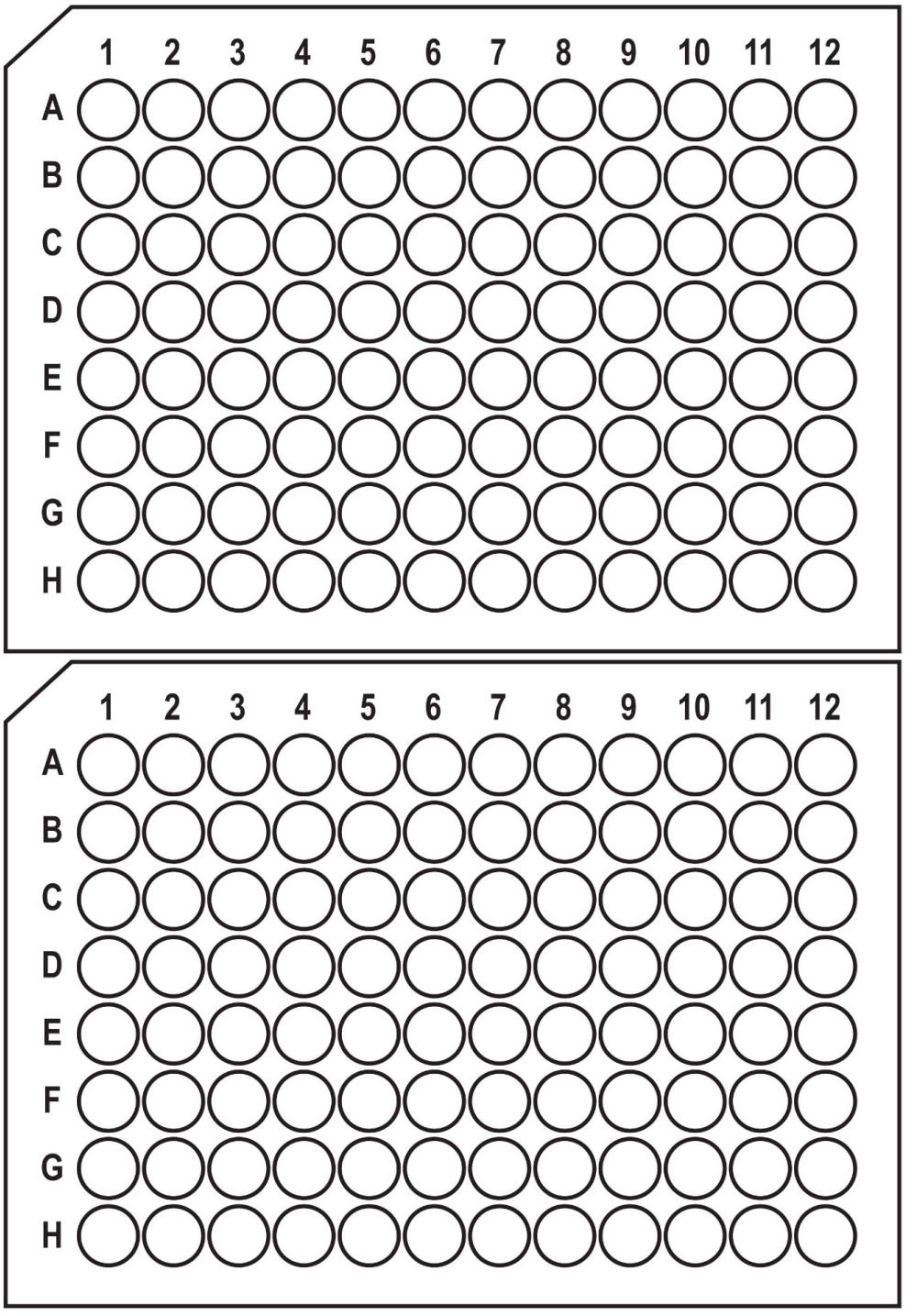


Figure 2. Plate diagram.