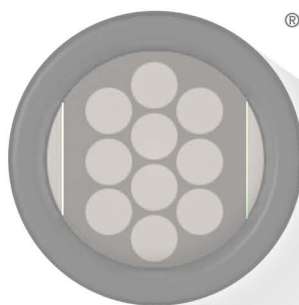


MSD[®] MULTI-SPOT Assay System

Mouse Isotyping Panel 1 Kit

IgA, IgG1, IgG2a, IgG2b, IgG3, IgM

T-PLEX[®]



1-Plate Kit

5-Plate Kit

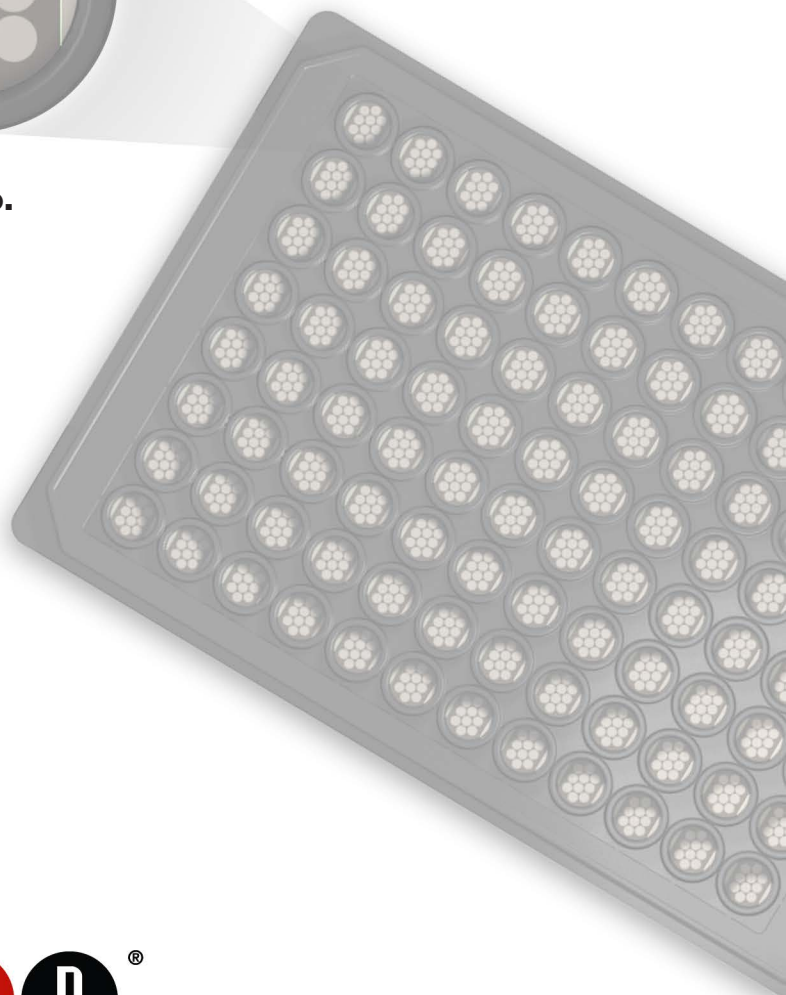
25-Plate Kit

Catalog No.

K15183B-1

K15183B-2

K15183B-4



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MSD MULTI-SPOT[®] Assays

Mouse Isotyping Panel 1 Kit

IgA, IgG1, IgG2a, IgG2b, IgG3, IgM

**FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

Meso Scale Discovery

A division of Meso Scale Diagnostics, LLC.

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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 in 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

MESO SCALE DISCOVERY® 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 T-PLEX® Mouse Isotyping Panel 1 Kit detects IgA, IgG1, IgG2a, IgG2b, IgG3, and IgM in a sandwich immunoassay format (Figure 1). T-PLEX products are provided with a 10-spot plate that has been precoated with capture antibodies on spatially distinct spots. The user adds the sample and a solution containing the detection antibodies conjugated with an electrochemiluminescent MSD SULFO-TAG™ 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 buffer that creates 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, which is proportional to the amount of analyte present in the sample and provides a quantitative measure of IgA, IgG1, IgG2a, IgG2b, IgG3, or IgM present in the sample.

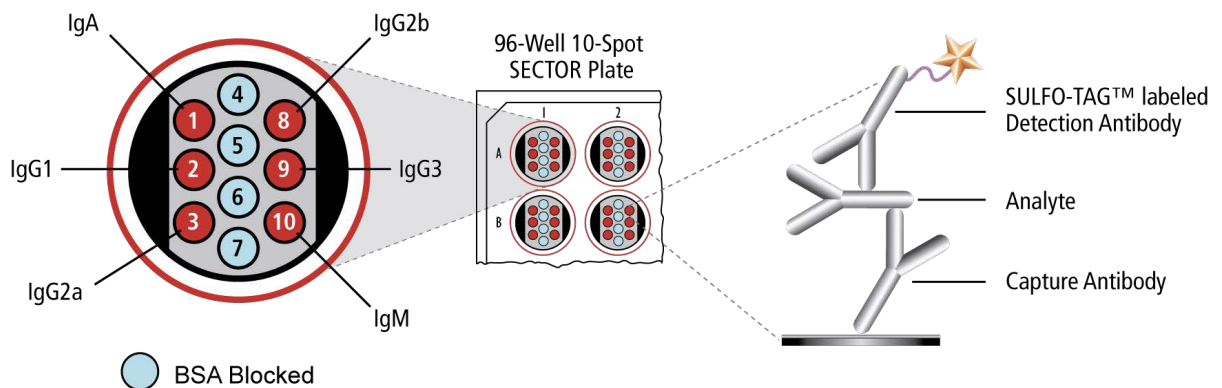


Figure 1. Multiplex plate spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

Standard Workflow for T-PLEX Mouse Isotyping Panel 1 Kit

An overview of the steps in the T-PLEX Mouse Isotyping Panel 1 Kit workflow with incubation durations is outlined below. This is an overview for planning purposes; for the full workflow, see *Protocol* on page 8.

Step	Incubation
Add Diluent 100	30 min.
Add samples and calibrators	2 hr.
Add detection antibody solution	2 hr.
Add read buffer	No incubation
Read plate	Read time is dependent on instrument model

Materials and Equipment

This section lists the components provided with the T-PLEX Mouse Isotyping Panel 1 Kit.

Reagents Supplied

Table 1. Reagents that are supplied with the T-PLEX Mouse Isotyping Panel 1 Kit

Reagent	Storage	Catalog No.	Size	Quantity Supplied		
				K15183B-1	K15183B-2	K15183B-4
96-Well 10-Spot Mouse Isotyping Panel 1 Plate	2–8 °C	N05183A-1	1 plate	1 Plate	5 Plates	25 Plates
SULFO-TAG Anti-mouse IgA Antibody (50X) ¹	2–8 °C	D22JJ-2	75 µL	1 vial	—	—
		D22JJ-3	375 µL	—	1 vial	5 vials
SULFO-TAG Anti-mouse IgG1 Antibody (50X) ¹	2–8 °C	D22JL-2	75 µL	1 vial	—	—
		D22JL-3	375 µL	—	1 vial	5 vials
SULFO-TAG Anti-mouse IgG2a Antibody (50X) ¹	2–8 °C	D22JM-2	75 µL	1 vial	—	—
		D22JM-3	375 µL	—	1 vial	5 vials
SULFO-TAG Anti-mouse IgG2b Antibody (50X) ¹	2–8 °C	D22JN-2	75 µL	1 vial	—	—
		D22JN-3	375 µL	—	1 vial	5 vials
SULFO-TAG Anti-mouse IgG3 Antibody (50X) ¹	2–8 °C	D22JO-2	75 µL	1 vial	—	—
		D22JO-3	375 µL	—	1 vial	5 vials
SULFO-TAG Anti-mouse IgGM Antibody (50X) ¹	2–8 °C	D22JP-2	75 µL	1 vial	—	—
		D22JP-3	375 µL	—	1 vial	5 vials
Mouse Isotyping Panel 1 Calibrator Blend	≤-70 °C	C0759-2	1 vial	1 vial	5 vials	25 vials
Diluent 100 ²	RT	R50AA-4	50 mL	1 bottle	1 bottle	—
		R50AA-2	200 mL	—	—	5 bottles
MSD GOLD™ Read Buffer B	RT	R60AM-1	18 mL	1 bottle	—	—
		R60AM-2	90 mL	—	1 bottle	5 bottles

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

²Aliquot and freeze after opening to avoid contamination.

dash (—) = not applicable

Additional Materials and Equipment

- Appropriately sized tubes for reagent preparation
- Polypropylene microcentrifuge tubes for preparing dilutions
- Liquid-handling equipment suitable for dispensing 10 to 150 μL /well into a 96-well microtiter plate
- Plate-washing equipment: automated plate washer or multichannel pipette
- Microtiter plate shaker (rotary) capable of shaking at 500–1,000 rpm
- MSD Wash Buffer (20X, 100 mL, catalog number R61AA-1) or phosphate-buffered saline (PBS) plus 0.05% Tween-20 (PBS-T) for plate washing
The standard protocol uses a minimum of 130 mL for a 96-well plate. Automated plate washers may need overage added to these volumes
- Adhesive plate seals
- Deionized water
- Vortex mixer

Safety

Use safe laboratory practices. Wear appropriate personal protective equipment, including 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 sheet(s) (SDS), which can be obtained from MSD Customer Service or at www.mesoscale.com.

Protocol

Best Practices

Read this product insert in its entirety before use. In addition, adhere to the following best practices:

Reagent Preparation

Do Not Mix Lots	Mixing or substituting reagents from different sources or different kit lots is not recommended. Lot information is provided in the lot-specific COA.
Thaw Reagents	Thaw reagents on wet ice and use them immediately.

Reagent Handling

Prepare in Polypropylene Tubes	Prepare calibrators and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution and mix by vortexing after each dilution.
Protect Reagents from Light	Avoid prolonged exposure of detection antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be protected from light except for direct sunlight.
Avoid Bubbles During Pipetting	Avoid bubbles in wells during all pipetting steps as they may lead to variable results. Bubbles introduced when adding read buffer may interfere with signal detection.
Use Reverse Pipetting	Use reverse pipetting when necessary to avoid the introduction of bubbles. If pipetting into an empty well, pipette gently to the bottom corner. Do not touch the bottom of the wells with the pipette tip when pipetting into the MSD plate.

Plate Handling

Protect Plate from Sunlight	Protect plates from direct sunlight.
Plate Shaking Guidelines	Plate shaking should use a rotary motion between 500–1,000 rpm.
Incubation Temperature	Assay incubation steps should be performed between 20–26 °C to achieve the most consistent signals between runs.
Tap Plate	Tap the plate on a paper towel to remove residual fluid after washing.
Incubation Extension	If an incubation step needs to be extended, leave the sample or detection antibody solution in the plate to keep the plate from drying out.

Plate Reading

Remove Plate Seal	Remove the plate seal before reading the plate.
Read Buffer at Room Temperature	Ensure that the read buffer is at room temperature (20–26 °C) before adding to the plate.
Do Not Shake Plate	Do not shake the plate after adding read buffer.
Time Intervals	Keep time intervals consistent between adding the read buffer and reading the plate to improve inter-plate precision. Prepare the MSD instrument to read a plate before adding read buffer. Unless otherwise directed, read the plate as soon as possible after adding the read buffer.
Results Above Curve	If the sample results are above the top of the calibration curve, dilute the samples and repeat the assay.

Reagent Preparation

Bring reagents to room temperature and refer to *Best Practices* on page 8 before beginning the protocol. Thaw the stock calibrator on ice.

Prepare Standards

MSD supplies a blended calibrator for the T-PLEX Mouse Isotyping Panel 1 Kit at a 100-fold higher concentration than the recommended highest calibrator. We recommend a 7-point standard curve with 4-fold serial dilution steps and a zero calibrator blank (Diluent 100). Each well requires 25 µL of calibrator.

To prepare 7 standard solutions plus a zero calibrator blank for up to 4 replicates:

1. Thaw the stock calibrator and keep it on ice.
2. Add calibrator to the Diluent 100 at room temperature to make the standard curve solutions according to the scheme below. Mix well between each step.

Calibrator Standard No.	Tube No.	Source of Calibrator	Volume of Calibrator (µL)	Diluent 100 (µL)	Total volume (µL)
1	1	Stock calibrator	10	990	1000
2	2	From tube 1	50	150	200
3	3	From tube 2	50	150	200
4	4	From tube 3	50	150	200
5	5	From tube 4	50	150	200
6	6	From tube 5	50	150	200
7	7	From tube 6	50	150	200
8 (zero calibrator)	8	—	0	200	200

Dash (—) = not applicable

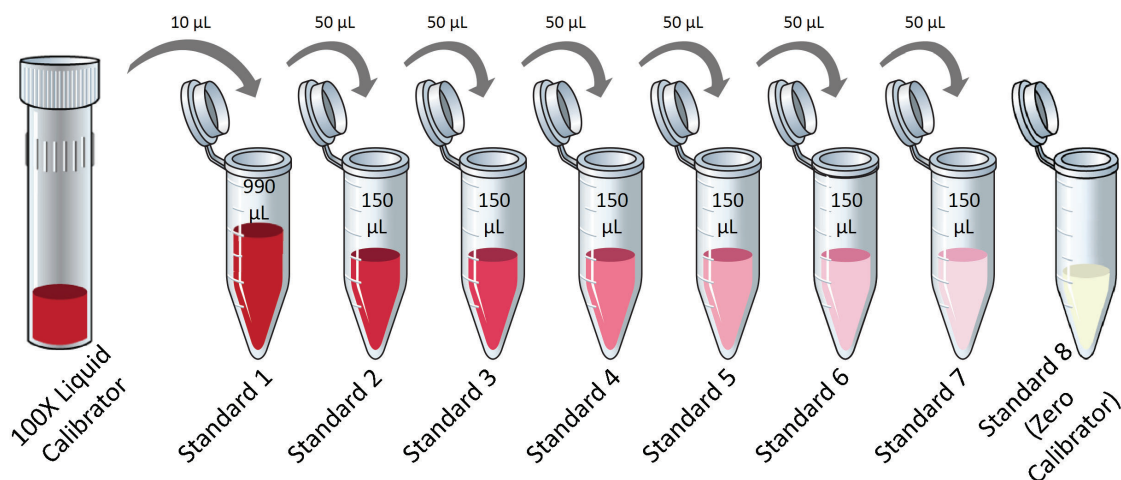


Figure 2. Dilution scheme for preparation of calibrator standards.

i Calibrators can also 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 the 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.

The concentrations of each calibrator after the dilutions are listed in Table 2. IgG2b Calibrator is at 4-fold lower concentrations to avoid saturation of instrument detection.

Table 2. Concentrations of each calibrator in the standard curve

Standard	All Isotypes (except IgG2b) (pg/mL)	IgG2b (pg/mL)	Dilution Factor
100X Stock	10,000,000	2,500,000	—
Standard 1	100,000	25,000	100
Standard 2	25,000	6,250	4
Standard 3	6,250	1,560	4
Standard 4	1,560	390	4
Standard 5	390	98	4
Standard 6	98	24	4
Standard 7	24	6	4
Standard 8	0	0	—

dash (—) = not applicable

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 1,000-fold to 100,000-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 (see *Reagents Supplied* on page 6).

Tissue Culture

Tissue culture supernatant samples may not require any dilution before being used in the T-PLEX Mouse Isotyping Panel 1 Kit. Samples from experimental conditions with extremely high levels of mouse Ig may require a dilution.

Prepare Detection Antibody Solution

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

For 1 plate, add the following detection antibodies to Diluent 100 for a total of 3,000 μ L:

- 2,640 μ L Diluent 100
- 60 μ L of 50X SULFO-TAG Anti-mouse IgA Antibody
- 60 μ L of 50X SULFO-TAG Anti-mouse IgG1 Antibody
- 60 μ L of 50X SULFO-TAG Anti-mouse IgG2a Antibody
- 60 μ L of 50X SULFO-TAG Anti-mouse IgG2b Antibody
- 60 μ L of 50X SULFO-TAG Anti-mouse IgG3 Antibody
- 60 μ L of 50X SULFO-TAG Anti-mouse IgM Antibody

i You may omit detection antibody for any analyte not being measured. In that case, add 60 μ L of Diluent 100 for each omitted antibody.

Prepare Wash Buffer

You can purchase MSD Wash Buffer (20X) stock solution from MSD (100 mL, catalog number R61AA-1). The working solution is 1X.

i PBS-T can be used instead of MSD Wash Buffer.

For one plate, combine:

- 15 mL of MSD Wash Buffer (20X)
- 285 mL of deionized water

Read Buffer

MSD GOLD Read Buffer B is provided ready to use. Do not dilute.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies 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., pre-wetting) is required.

Assay Protocol

This section describes the assay protocol. Complete *Reagent Preparation* on page 9 before beginning this assay protocol.

STEP 1: Add Diluent 100

- ❑ 1. Add 25 μL of Diluent 100 to each well.
Seal the plate with an adhesive plate seal.
Incubate 30 minutes at room temperature with vigorous shaking (500–1,000 rpm).

STEP 2: Add Samples and Calibrators

- ❑ 2. Add 25 μL of sample (standards or unknowns) to each well.
Seal the plate with an adhesive plate seal.
Incubate 2 hours at room temperature with vigorous shaking (500–1,000 rpm).

i You can prepare detection antibody solution during the incubation.

STEP 3: Wash and Add Detection Antibody Solution

- ❑ 3. Wash the plate 3 times with 300 μL /well of 1X MSD Wash Buffer or PBS-T.
- ❑ 4. Add 25 μL of 1X Detection Antibody Solution to each well.
Seal the plate with an adhesive plate seal.
Incubate 2 hours at room temperature with vigorous shaking (500–1,000 rpm).

STEP 4: Wash and Read

- ❑ 5. Wash the plate 3 times with 300 μL /well of 1X MSD Wash Buffer or PBS-T.
- ❑ 6. Add 150 μL of MSD GOLD Read Buffer B to each well.
- ❑ 7. Read the plate on an MSD instrument. Incubation in read buffer is not required before reading the plate.

Analysis of Results

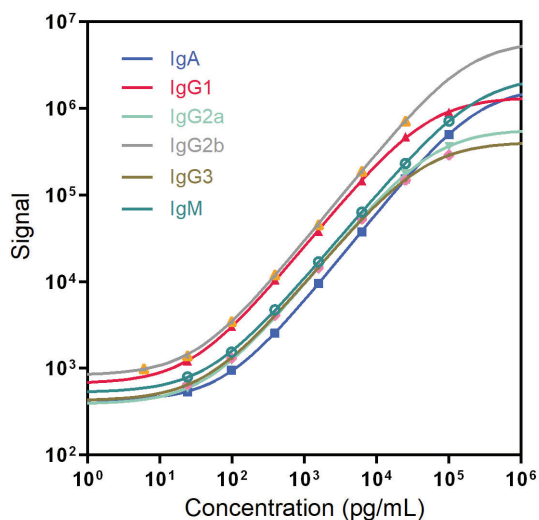
When analyzing results, note the following:

- Run at least one set of calibrators in duplicate to generate the standard curve.
- The standard curve is modeled using least squares fitting algorithms so that signals from the calibrators can be used to calculate the concentration of analyte in the samples.
- The assays have a wide dynamic range (3–4 logs) which allows accurate quantification without the need for dilution in many cases.
- The analysis software uses 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.

Assay Characteristics

Typical Standard Curve

The following standard curves are examples 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			IgG1			IgG2a		
Concentration (pg/mL)	Average Signal	%CV	Concentration (pg/mL)	Average Signal	%CV	Concentration (pg/mL)	Average Signal	%CV
100,000	500,974	4.4	100,000	901,279	5.4	100,000	364,097	7.7
25,000	154,010	2.7	25,000	471,145	5.2	25,000	175,595	3.9
6,250	37,648	4.9	6,250	146,478	6.2	6,250	55,539	4.8
1,563	9,588	0.5	1,563	38,009	1	1,563	13,715	1.1
391	2,539	1.1	391	10,504	2.2	391	4,047	5.4
98	950	2.5	98	3,026	0.4	98	1,239	4.2
24	537	4.6	24	1,218	5.5	24	590	2.4
0	404	0.7	0	652	5.2	0	369	1.3

IgG2b			IgG3			IgM		
Concentration (pg/mL)	Average Signal	%CV	Concentration (pg/mL)	Average Signal	%CV	Concentration (pg/mL)	Average Signal	%CV
25,000	720,105	10.3	100,000	290,603	4.6	100,000	714,777	5.7
6,250	189,782	9.6	25,000	147,787	0.8	25,000	231,709	2.1
1,563	45,890	7.6	6,250	53,077	1.6	6,250	63,735	1.9
391	12,129	6.8	1,563	14,566	3.9	1,563	16,954	3.6
98	3,491	6.3	391	4,097	2.4	391	4,756	4.4
24	1,405	6.7	98	1,311	1.1	98	1,541	1.8
6	997	31.5	24	650	1.3	24	795	3.1
0	717	9	0	430	3.1	0	517	0.8

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 runs.

Table 3. LLOD for each analyte in the T-PLEX Mouse Isotyping Panel 1 Kit.

	IgA	IgG1	IgG2a	IgG2b	IgG3	IgM
LLOD (pg/mL)	15	3.8	8.7	3.0	8.2	46.8

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 on a 7-spot plate. Calibrator curve was prepared in Diluent 100.

$$\% \text{ recovery} = \frac{\text{measured concentration}}{\text{expected concentration}} \times 100$$

Table 4. Spike and recovery measurements of different sample types in the T-PLEX Mouse Isotyping Panel 1 Kit

	IgA				IgG1				IgG2a			
	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	%Recovery	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	%Recovery	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	%Recovery
Tissue Culture Media	0	0	—	—	0	0	—	—	0	0	—	—
	2,000	1,554	4.8	78	2,000	1,414	2.5	71	2,000	1,525	13.6	76
	20,000	16,382	7.4	82	20,000	15,560	1.7	78	20,000	15,881	11.3	79
	IgG2b				IgG3				IgM			
	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	%Recovery	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	%Recovery	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	%Recovery
Tissue Culture Media	0	0	—	—	0	0	—	—	0	0	—	—
	2,000	1,614	10.5	81	2,000	1,653	4.9	83	2,000	1,613	6.1	81
	20,000	17,373	4.7	87	20,000	15,485	6.9	77	20,000	16,188	11.7	81

dash (—) = not applicable

Dilution Linearity

Tissue culture media was spiked with calibrators, diluted with Diluent 100, then tested on a 7-spot plate. 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).

$$\% \text{ recovery} = \frac{\text{measured concentration}}{\text{expected concentration}} \times 100$$

The results for tissue culture media are shown in Table 1.

Table 5. Analyte percent recovery at various dilutions in Tissue Culture Media.

		IgA			IgG1			IgG2a		
Sample Type	Fold Dilution	Conc. (pg/mL)	Conc. % CV	% Recovery	Conc. (pg/mL)	Conc. % CV	% Recovery	Conc. (pg/mL)	Conc. % CV	% Recovery
Tissue Culture Media	1	5,558	7.6	—	6,013	3.5	—	5,846	3.4	—
	2	5,543	7.3	100	5,416	2.4	90	5,679	6.8	97
	4	5,431	9.3	98	5,520	8.6	102	5,428	6.7	96
	8	5,457	15.7	100	5,543	2.3	100	5,331	5.8	98
		IgG2b			IgG3			IgM		
Sample Type	Fold Dilution	Conc. (pg/mL)	Conc. % CV	% Recovery	Conc. (pg/mL)	Conc. % CV	% Recovery	Conc. (pg/mL)	Conc. % CV	% Recovery
Tissue Culture Media	1	5,711	7.2	—	5,658	3.8	—	4,959	4.4	—
	2	5,481	7.6	96	5,402	3.4	95	5,532	8.7	112
	4	5,497	4.9	100	5,840	5.1	108	5,618	8.5	102
	8	5,659	9.6	103	5,331	9.6	91	4,930	4.7	88

dash (—) = not applicable

Pooled serum, EDTA plasma, and heparin plasma were diluted with Diluent 100. The results are shown in Table 6.

Table 6. Analyte percent recovery at various dilutions in Serum, EDTA Plasma, and Heparin Plasma.

		IgA			IgG1			IgG2a		
Sample Type	Fold Dilution	Conc. (pg/mL)	Conc. % CV	% Recovery	Conc. (pg/mL)	Conc. % CV	% Recovery	Conc. (pg/mL)	Conc. % CV	% Recovery
Serum	10,000	51	17.0	—	640	1.1	—	350	2.3	—
	20,000	51	2.2	101	829	6.9	130	458	3.6	131
	40,000	58	2.4	113	851	6.2	103	547	6.2	119
	80,000	57	4.9	97	851	3.3	100	584	1.9	107
EDTA Plasma	10,000	36	9.9	—	599	11.8	—	362	7.7	—
	20,000	39	5.2	107	713	2.2	119	476	3.5	132
	40,000	41	3.8	106	728	9.2	102	586	3.7	123
	80,000	46	1.2	111	707	9.9	97	655	6.9	112
Heparin Plasma	10,000	79	4.1	—	778	5.9	—	427	5.9	—
	20,000	82	10.7	103	857	10.3	110	593	4.0	139
	40,000	91	3.1	111	978	3.9	114	801	4.3	135
	80,000	94	9.9	104	887	7.2	91	867	1.9	108

		IgG2b			IgG3			IgM		
Sample Type	Fold Dilution	Conc. (pg/mL)	Conc. % CV	% Recovery	Conc. (pg/mL)	Conc. % CV	% Recovery	Conc. (pg/mL)	Conc. % CV	% Recovery
Serum	10,000	180	12.7	—	109	5.6	—	223	3.0	—
	20,000	196	9.3	108	115	3.0	105	209	3.2	94
	40,000	207	1.3	106	121	5.7	105	216	3.2	104
	80,000	211	7.3	102	121	3.0	100	222	5.5	103
EDTA Plasma	10,000	197	7.2	—	107	3.9	—	164	12.9	—
	20,000	233	9.7	118	128	2.0	120	172	10.9	105
	40,000	272	3.1	117	134	3.5	105	173	9.4	101
	80,000	281	9.3	103	139	3.9	103	165	8.4	95
Heparin Plasma	10,000	281	8.9	—	179	4.9	—	216	10.7	—
	20,000	347	9.6	124	186	7.7	104	211	17.8	98
	40,000	379	3.0	109	194	7.2	104	208	12.7	99
	80,000	388	13.7	102	207	4.9	106	203	11.0	97

dash (—) = not applicable

Specificity

The specificity of the new mouse isotyping assays was evaluated by measuring individual spiked immunoglobulin calibrators at 6.25 ng/mL in each assay. All cross-reactivity was less than 0.1% with the exception of the IgG3 assay, which cross-reacts with IgM at 0.5%.

Additional Information

Appendix A: Recommended Plate Washer Parameters

Create a program for your automated plate washer with the optimal settings before starting your assay. Example settings for a typical (MSD-recommended) wash program are shown below for a common plate washer (Biotek Model 405 LS, Table 7).

Table 7. Parameters for customized programs on the Biotek 405 LS microplate washer

Wash Program Parameters		Typical Wash Program Settings
	Plate type	96
CYCLES	Wash cycles	3
ASPIRATION	Aspirate Type	TOP
	Travel Rate	1 (4.1% 1.0 mm/second)
	Aspirate Delay	0500 milliseconds
	Aspirate X-Position	-35
	Aspirate Y-Position	-35
	Aspirate Height	22
	Secondary Aspirate?	NO
DISPENSE	Dispense Rate	05
	Dispense Volume	0300 µL/well
	Vacuum Delay Volume	0300 µL/well
	Dispense X-Position	00 (0.000 mm)
	Dispense Y-Position	00 (0.000 mm)
	Dispense Height	120 (15.245 mm)
OPTS PRE	Wash Pre dispense?	NO
	Bottom Wash?	NO
MIDCYC	Wash Shake?	NO
	Wash Soak?	NO
	Home Carrier?	NO
	Between Cycle Pre Dispense?	NO
POST	Final Aspirate?	YES
	Aspirate Type	TOP
	Travel Rate	3
	Final Aspirate Delay	0500 milliseconds
	Final Aspirate X-Position	-35 (1.600 mm)
	Final Aspirate Y-Position	-35 (1.600 mm)
	Final Aspirate Height	22
	Secondary Aspirate?	YES
	Final Aspirate Secondary X-Position	35 (1.600 mm)
	Final Aspirate Secondary Y-Position	35 (1.600 mm)
Final Aspirate Secondary Height	22	

Summary Protocol

Sample and Reagent Preparation

- Bring all reagents to room temperature.
- Prepare calibration solutions in Diluent 100 using the supplied calibrator.
- Dilute the stock calibrator 100-fold in Diluent 100.
- Perform a series of 4-fold dilution steps and prepare a zero calibrator.
- Dilute samples in Diluent 100 before adding to the plate, if required.
- Prepare combined detection antibody solution by diluting each 50X detection antibody 50-fold in Diluent 100.

STEP 1: Add Diluent 100

- 1. Add 25 μ L of Diluent 100 to each well.
Seal the plate with an adhesive plate seal.
Incubate 30 minutes at room temperature with vigorous shaking (500–1,000 rpm).

STEP 2: Add Samples and Calibrators

- 2. Add 25 μ L of sample (standards or unknowns) to each well.
Seal the plate with an adhesive plate seal.
Incubate 2 hours at room temperature with vigorous shaking (500–1,000 rpm).

STEP 3: Wash and Add Detection Antibody Solution

- 3. Wash the plate 3 times with 300 μ L/well of 1X MSD Wash Buffer or PBS-T.
- 4. Add 25 μ L of 1X Detection Antibody Solution to each well.
Seal the plate with an adhesive plate seal.
Incubate 2 hours at room temperature with vigorous shaking (500–1,000 rpm).

STEP 4: Wash and Read

- 5. Wash the plate 3 times with 300 μ L/well of 1X MSD Wash Buffer or PBS-T.
- 6. Add 150 μ L of MSD GOLD Read Buffer B to each well.
- 7. Read the plate on an MSD instrument. Incubation in read buffer is not required before reading the plate.

Plate Diagram

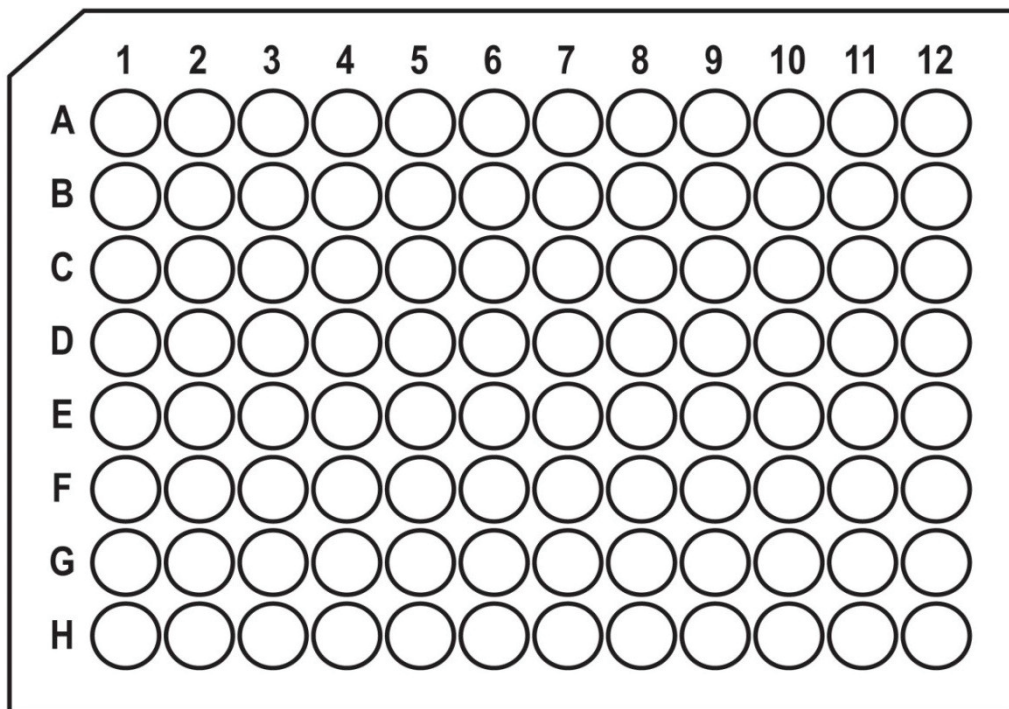


Figure 3. Plate diagram.

Recommended Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL-01		Sample-01		Sample-09		Sample-17		Sample-25		Sample-33	
B	CAL-02		Sample-02		Sample-10		Sample-18		Sample-26		Sample-34	
C	CAL-03		Sample-03		Sample-11		Sample-19		Sample-27		Sample-35	
D	CAL-04		Sample-04		Sample-12		Sample-20		Sample-28		Sample-36	
E	CAL-05		Sample-05		Sample-13		Sample-21		Sample-29		Sample-37	
F	CAL-06		Sample-06		Sample-14		Sample-22		Sample-30		Sample-38	
G	CAL-07		Sample-07		Sample-15		Sample-23		Sample-31		Sample-39	
H	CAL-08		Sample-08		Sample-16		Sample-24		Sample-32		Sample-40	

Figure 4. Recommended plate layout for the assay. Each sample and calibrator is measured in duplicate in side-by-side wells.

Catalog Numbers

Table 8. Catalog numbers associated with the T-PLEX Mouse Isotyping Panel 1 Kit

Kit Name	SECTOR Plate		
	1-Plate Kit	5-Plate Kit	25-Plate Kit
T-PLEX Mouse Isotyping Panel 1 Kit	K15183B-1	K15183B-2	K15183B-4

Table 9. Instrument compatibility for plate type

Plate Type	Instrument Compatibility
SECTOR™ Plate	MESO® SECTOR S 600, MESO SECTOR® S 600MM, MESO QuickPlex® SQ 120, MESO QuickPlex SQ 120MM

i Ensure the plate type is compatible with your MSD instrument.
