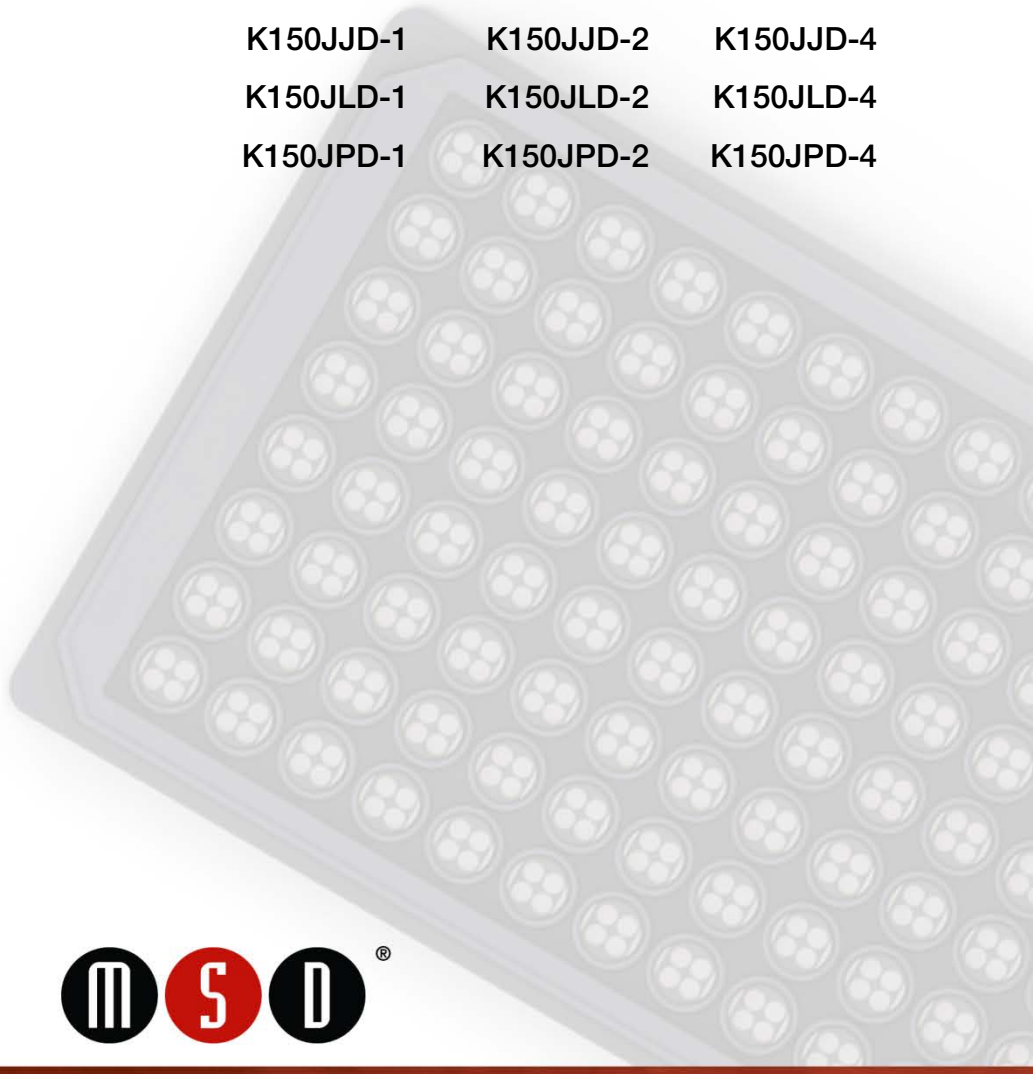
MSD® MULTI-ARRAY Assay System

Human/NHP Isotyping Kits

Multiplex Kit	1 Plate	5 Plates	25 Plates
Isotyping Panel 1 (Human/NHP)	K15203D-1	K15203D-2	K15203D-4
Singleplex Kits			
Human/NHP IgA	K150JJD-1	K150JJD-2	K150JJD-4
Human/NHP IgG	K150JLD-1	K150JLD-2	K150JLD-4
Human/NHP IgM	K150JPD-1	K150JPD-2	K150JPD-4



MSD Assay Development Tools

Human/NHP Isotyping Kits

For use with human and non-human primate (NHP) samples.

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®
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Ordering Information

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Introduction

Immunoglobulins (Ig) are critical components of the immune system and play a major role in eliciting immune response against pathogens. Five heavy-chain isotypes (IgA, IgD, IgE, IgG, and IgM) mediate specific biological functions,[1] including responses to pathogens and immunological diseases such as allergy and autoimmune disorder.[2]

IgA has two subclasses (IgA1 and IgA2) and exists in both monomeric and dimeric forms. It is approximately 15% of the total serum Ig content.[3] Secretory IgA is a dimer that provides the primary defense against localized infections. High levels of IgA are often found in patients with chronic infections such as cirrhotic liver disease.[4]

IgG, a monomer, is the dominant immunoglobulin class present in serum. There are four subclasses of IgG, namely IgG1, IgG2, IgG3, and IgG4.[5] IgG is commonly used as a diagnostic tool for humoral immunodeficiencies, autoimmune diseases, liver diseases, chronic inflammatory diseases, haematological disorders, infections, and lymphoid malignancies.[6,7]

IgM is a pentamer that comprises approximately 10% of serum immunoglobulin content.[8] IgM level is often increased in patients with infectious diseases including primary biliary cirrhosis, infectious mononucleosis, cytomegalovirus infection, and tuberculosis.[9]

MSD offers individual isotyping kits for the measurement of IgA, IgG, and IgM in human and non-human primate samples. In addition, a multiplex panel is available that measures all three analytes in a single well. The kits mentioned in this product insert have been tested with human, cynomolgus monkey, and rhesus monkey sera samples.

Principle of the Assay

MSD isotyping kits provide a rapid and convenient method for measuring the levels of different immunoglobulins within a single, small-volume sample. The assays in the Human/NHP Isotyping Kits are sandwich immunoassays. MSD provides a plate pre-coated with capture antibodies on independent and well-defined spots in the layout shown below. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) 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 detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

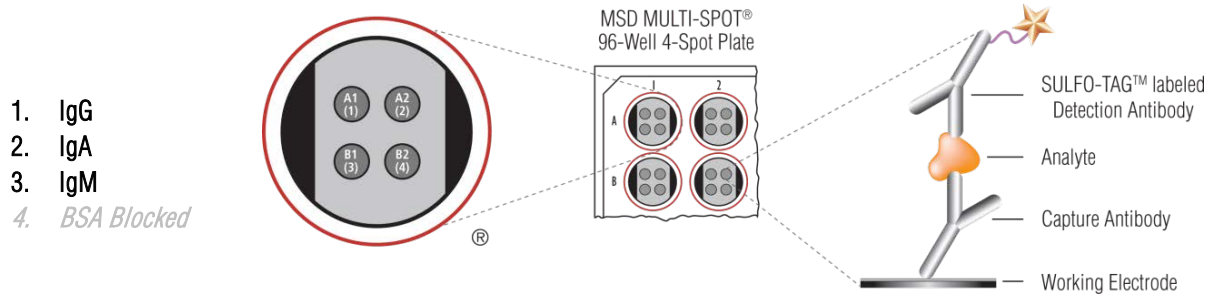


Figure 1. Spot diagram showing placement of analyte capture antibodies for the Isotyping Panel 1 (Human/NHP) Kit. Singleplex kits are also provided with the above plate, but with kit-specific detection antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

Reagents Supplied

Human/NHP Isotyping assays are available as a multiplex kit and as single assay kits. All kits share common reagents except for the detection antibodies. Multiplex kits include detection antibodies for all three analytes, while the single analyte kits include only the analyte-specific detection antibody.

Reagents Supplied With All Kits

Reagent	Storage	Catalog #	Size	Quantity Supplied			Description
				1-Plate Kit	5-Plate Kit	25-Plate Kit	
MULTI-SPOT®, 96-well Isotyping Panel 1 (Human/NHP) Plate	2–8°C	N45203A-1	4-spot	1	5	25	96-well plate, foil sealed, with desiccant.
Isotyping Panel 1 (Human/NHP) Calibrator Blend	≤-70°C	C0203-2	1 vial	1 vial	5 vials	25 vials	Blend of three recombinant human proteins (IgA, IgG, and IgM) in diluent and frozen.
Diluent 100	2–8°C	R50AA-2	200 mL	1 bottle			Diluent for samples, calibrator, and detection antibodies; contains PBS, blockers, and preservatives.
		R50AA-3	1000 mL		1 bottle	5 bottles	
Blocker A Kit	RT	R93AA-2	250 mL	1 kit	1 kit	5 kits	Cocktail of proteins in a PBS-based buffer optimized for use with MSD assays. Blocker A Kit contains dry Blocker A powder and a 5X solution of phosphate buffer.
Read Buffer T (4X)	RT	R92TC-3	50 mL	1 bottle	1 bottle	5 bottles	Buffer to catalyze the electro-chemiluminescence reaction.

Kit-Specific Components

The Isotyping Panel 1 (Human/NHP) Kit includes all three antibodies mentioned in the table below. Single analyte kits are supplied with specific detection antibodies as indicated in the table.

Detection Antibodies*	Storage	Part #	Size	Quantity Supplied			Description	Kit
				1-Plate Kit	5-Plate Kit	25-Plate Kit		
SULFO-TAG Anti-Hu/ NHP IgG Antibody (50X)	2–8°C	D20JL-2	75 µL	1			SULFO-TAG–conjugated antibody	Human/NHP IgG Kit
		D20JL-3	375 µL		1	5		
SULFO-TAG Anti-Hu/ NHP IgA Antibody (50X)	2–8°C	D20JJ-2	75 µL	1			SULFO-TAG–conjugated antibody	Human/NHP IgA Kit
		D20JJ-3	375 µL		1	5		
SULFO-TAG Anti-Hu/ NHP IgM Antibody (50X)	2–8°C	D20JP-2	75 µL	1			SULFO-TAG–conjugated antibody	Human/NHP IgM Kit
		D20JP-3	375 µL		1	5		

*The Isotyping Panel 1 (Human/NHP) Kit includes all three antibodies.

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing serial dilutions
- ❑ Liquid handling equipment for 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
- ❑ Microtiter plate shaker (rotary) capable of shaking at 300–1000 rpm
- ❑ Phosphate-buffered saline plus 0.05% Tween-20 for plate washing or MSD Wash Buffer, catalog # R61AA-1
- ❑ Adhesive plate seals
- ❑ Deionized water

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit 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 safety data sheet (SDS), which can be obtained from MSD Customer Service.

Best Practices and Technical Hints

- Do not mix or substitute reagents from different sources or different kit lots.
- Dilute calibrators and samples in polypropylene microcentrifuge tubes; use a fresh pipette tip for each dilution; vortex after each dilution before proceeding.
- Avoid prolonged exposure of detection antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be shielded from light except for direct sunlight.
- Avoid bubbles in wells at all pipetting steps. Bubbles may lead to variable results; bubbles introduced when adding read buffer may interfere with signal detection.
- Use reverse pipetting when necessary to avoid introduction of bubbles, and for empty wells, pipette to the bottom corner.
- Shaking should be vigorous with a rotary motion between 300 and 1000 rpm.
- When using an automated plate washer, rotating the plate 180 degrees between wash steps may improve assay precision.
- Gently tap the plate to remove residual fluid after washing.
- Read buffer should be at room temperature when added to the plate.
- Keep time intervals consistent between adding read buffer and reading the plate to improve inter-plate precision. Unless otherwise directed, read plate as soon as practical after adding read buffer.
- No shaking is necessary after adding read buffer.
- If an incubation step needs to be extended, avoid letting the plate dry out by keeping sample or detection antibody solution in the plate.
- Remove plate seals prior to reading the plate.
- If assay results are above the top of the calibration curve, dilute samples, and repeat the assay.
- When running a partial plate, seal the unused sectors (see sector map in instrument and software manuals) to avoid contaminating unused wells. (Remove all seals before reading.) Partially used plates may be sealed and stored up to 30 days at 2–8°C in the original foil pouch with desiccant.
- You may adjust volumes proportionally when preparing reagents.

Reagent Preparation

Bring all reagents to room temperature.

Prepare Blocker A Solution

Follow the instructions included in the Blocker A Kit.

Prepare Calibrator Dilutions

MSD supplies a multi-analyte blended calibrator for the Human/NHP Isotyping Kits at 20-fold higher concentration than the recommended highest calibrator. We recommend a 7-point calibration curve with 4-fold serial dilution steps and a zero calibrator blank. Thaw the stock calibrator and keep on ice, then add to diluent at room temperature to make the calibration curve solutions.

Calibrator	IgG (pg/mL)	IgA (pg/mL)	IgM (pg/mL)	Dilution Factor
Stock Calibrator	4 000 000	4 000 000	4 000 000	
Calibrator-01	200 000	200 000	200 000	20
Calibrator-02	50 000	50 000	50 000	4
Calibrator-03	12 500	12 500	12 500	4
Calibrator-04	3 125	3 125	3 125	4
Calibrator-05	781	781	781	4
Calibrator-06	195	195	195	4
Calibrator-07	49	49	49	4
Calibrator-08	0	0	0	n/a

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

1. Prepare the highest calibrator by adding 15 µL of stock calibrator to 285 µL of Diluent 100. Mix well.
2. Prepare the next calibrator by transferring 60 µL of the highest calibrator to 180 µL of Diluent 100. Mix well. Repeat 4-fold serial dilutions 5 additional times to generate 7 calibrators.
3. Use Diluent 100 as the blank.

Dilute Samples

Dilute samples with Diluent 100. For human and non-human primate sera, MSD recommends a 250 000-fold dilution.

For example, to dilute 250 000-fold:

1) Add 10 µL of sample to 990 µL of Diluent 100 (100-fold dilution)
2) Add 10 µL of the diluted sample from step 1 to 990 µL of Diluent 100 (100-fold dilution)
3) Add 40 µL of the diluted sample from step 2 to 960 µL of Diluent 100 (25-fold dilution).

Prepare Detection Antibody Solution

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

Isotyping Panel 1 (Human/NHP) Kit:

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-Hu/NHP IgG Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-Hu/NHP IgA Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-Hu/NHP IgM Antibody
- ❑ 2820 µL of Diluent 100

Singleplex Kits

For 1 plate, add 60 µL of the supplied 50X detection antibody to 2940 µL of Diluent 100.

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 keep excess diluted read buffer in a tightly sealed container at room temperature for up to 1 month.

Prepare MSD Plate

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

Protocol

1. **Block plate:** Add 150 µL of Blocker A solution to each well. Seal the plate with an adhesive plate seal and incubate for 30 minutes with shaking at room temperature.

2. **Wash and Add Sample:** Wash the plate 3 times with at least 150 µL/well of PBS-T. Add 25 µL of diluted sample or calibrator per well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 2 hours.

3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with at least 150 µL/well of PBS-T. Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 2 hours.

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

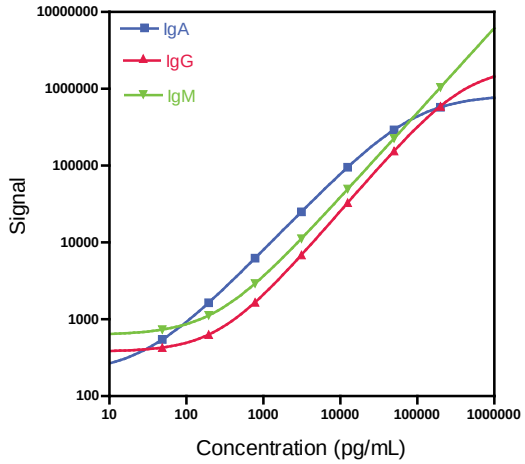
Analysis of Results

The calibration curve is modeled using least squares fitting algorithms to calculate the concentration of analyte in the samples using signals from the calibrators. The assays have a wide dynamic range (4 logs), which allows accurate quantification of samples without the need for multiple dilutions or repeated testing. The data displayed below were generated by DISCOVERY WORKBENCH® analysis software using a 4-parameter, logistic curve-fitting model (sigmoidal dose-response) with a $1/Y^2$ weighting function. The weighting function provides a better fit of data over a wide dynamic range, particularly at the low end of the calibration curve.

Best quantification of unknown samples will be achieved by generating a calibration curve for each plate using a minimum of 2 replicates at each calibrator level.

Typical Data

The following calibration curve graph illustrates the dynamic range of the assays. Actual signals will vary.



IgA		
Conc. (pg/mL)	Average Signal	%CV
0	190	5.2
49	545	14.3
195	1 646	42.3
781	6 244	3.3
3 125	25 019	10.2
12 500	95 347	7.5
50 000	292 294	3.2
200 000	575 772	0.5

IgG		
Conc. (pg/mL)	Average Signal	%CV
0	330	8.4
49	429	8.7
195	632	19.5
781	1 662	8.8
3 125	6 870	3.7
12 500	32 879	4.4
50 000	154 775	4.9
200 000	591 849	3.0

IgM		
Conc. (pg/mL)	Average Signal	%CV
0	552	6.5
49	734	5.9
195	1 123	2.5
781	2 919	0.2
3 125	11 210	7.4
12 500	49 490	8.9
50 000	226 219	12.0
200 000	1 042 000	4.2

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration corresponding to a signal 2.5 standard deviations above the background (zero calibrator).

	IgG	IgA	IgM
Average LLOD (pg/mL)	38	3.0	15

Parallelism

To assess parallelism, normal human and non-human primate sera samples were diluted 100 000-fold, 250 000-fold, 500 000-fold, and 1 000 000-fold before testing. Percent recovery at each dilution was calculated by dividing the dilution-adjusted concentration by the expected concentration, i.e., the dilution-adjusted concentration at 250 000-fold dilution.

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

		IgG		IgA		IgM	
Sample Type	Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Human Serum (N=5)	100 000	89	78–95	97	96–98	97	85–105
	500 000	98	90–104	97	93–101	108	99–118
	1 000 000	87	80–92	90	80–96	114	104–120
NHP Serum (cynomolgus monkey) (N=5)	100 000	102	97–107	101	91–110	97	94–100
	500 000	84	92–109	94	86–100	109	96–118
	1 000 000	71	65–78	78	69–82	106	95–113

Tested Samples

Normal human and non-human primate sera samples from a commercial source were diluted 250 000-fold and tested. Results for each sample set are displayed below. Concentrations are corrected for sample dilution.

		Concentration (mg/mL)		
	Samples	IgG	IgA	IgM
Human Serum	Sample 1	11.0	2.11	0.87
	Sample 2	14.8	2.56	1.31
	Sample 3	9.7	1.51	3.04
	Sample 4	15.7	3.40	2.01
	Sample 5	23.9	1.31	0.61
NHP Serum (cynomolgus monkey)	Sample 1	6.2	1.33	1.05
	Sample 2	5.4	0.37	0.15
	Sample 3	8.3	1.07	1.38
	Sample 4	5.3	1.30	0.98
	Sample 5	5.2	0.63	0.07

Specificity

To assess specificity, each assay in the panel was tested individually. Nonspecific binding was less than 0.5% for all assays.

Assay Components

Calibrators

The calibrator blend used in the Human/NHP Isotyping Kits consists of recombinant human IgA, IgG, and IgM proteins expressed in CHO cells.

Antibodies

The capture and detection antibodies used in the Human/NHP Isotyping Kits are listed below. They cross-react with human, cynomolgus monkey, and rhesus monkey samples.

	Source Species		
Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
IgG	Mouse Monoclonal	Mouse Monoclonal	A
IgA	Mouse Monoclonal	Mouse Monoclonal	A
IgM	Mouse Monoclonal	Mouse Monoclonal	A

References

1. Litman GW, et al. Phylogenetic diversification of immunoglobulin genes and the antibody repertoire. Mol Biol Evol. 1993;10:60-72.
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7. Dispenzieri A, et al. Retrospective cohort study of 148 patients with polyclonal gammopathy. Mayo Clin Proc. 2001;76:476-87.
8. Kindt TJ, et al. (2007). Kuby Immunology. *Antigens and Antibodies.* (pp. 76-106). New York, W.H. Freeman.
9. Lerner AM, et al. IgM serum antibodies to Epstein-Barr virus are uniquely present in a subset of patients with the chronic fatigue syndrome. In Vivo. 2004;18:101-6.

Summary Protocol

Human/NHP Isotyping Kits

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human/NHP Isotyping assays.

Sample and Reagent Preparation

Thaw calibrator on ice.

Bring all reagents to room temperature.

Prepare 7 calibration solutions using the supplied calibrator:

- Dilute the stock calibrator 20-fold in Diluent 100.
- Perform a series of 4-fold dilution steps and prepare a zero calibrator.

Dilute samples 250 000-fold in Diluent 100 before adding to the plate.

Prepare detection antibody solution by diluting stock detection antibody 50-fold in Diluent 100.

Prepare 2X Read Buffer T by diluting stock 4X Read Buffer T 2-fold with deionized water.

Step 1: Block Plate

Add 150 µL/well of Blocker A solution.

Incubate at room temperature with shaking for 30 minutes.

Step 2: Wash and Add Sample

Wash plate 3 times with at least 150 µL/well of PBS-T.

Add 25 µL/well of diluted sample (calibrators or unknowns).

Incubate at room temperature with shaking for 2 hours.

Step 3: Wash and Add Detection Antibody Solution

Wash plate 3 times with at least 150 µL/well of PBS-T.

Add 25 µL/well of 1X detection antibody solution.

Incubate at room temperature with shaking for 2 hours.

Step 4: Wash and Read Plate

Wash plate 3 times with at least 150 µL/well of PBS-T.

Add 150 µL/well of 2X Read Buffer T.

Read plate on MSD imager.

Related Products

MSD offers a range of immunoassay kits and reagents for isotyping applications. The catalog numbers for some of the products are provided below. Visit www.mesoscale.com for a complete list of available products.

	Product Name	Catalog Number
Assay Kits	Isotyping Panel 1 (Human/NHP) Kit	K15203D
	Human/NHP IgA Kit	K150JJD
	Human/NHP IgG Kit	K150JLD
	Human/NHP IgM Kit	K150JPD
Labeled Reporters	SULFO-TAG–Conjugated Anti-Hu/NHP IgA Antibody	D20JJ
	SULFO-TAG–Conjugated Anti-Hu/NHP IgG Antibody	D20JL
	SULFO-TAG–Conjugated Anti-Hu/NHP IgM Antibody	D20JP
	SULFO-TAG–Conjugated Anti-Hu/NHP Kappa Light Chain Antibody	D20TF
	SULFO-TAG–Conjugated Anti-Hu/NHP Lambda Light Chain Antibody	D20QG
	SULFO-TAG–Conjugated Streptavidin	R32AD

Ordering Information

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Plate Diagram