MSD® MULTI-ARRAY Assay System

Human/NHP Isotyping Kits

Multiplex Kit	1 Plate	5 Plates	25 Plates
Human/NHP Isotyping Panel 1 SECTOR™	K15770U-1	K15770U-2	K15770U-4
Human/NHP Isotyping Panel 1 QuickPlex®	K15770U-21	K15770U-22	K15770U-24

Singleplex Kits			
Human/NHP IgA v2 Kit SECTOR	K150AYNU-1	K150AYNU-2	K150AYNU-4
Human/NHP IgA v2 Kit QuickPlex	K150AYNU-21	K150AYNU-22	K150AYNU-24
Human/NHP IgG v2 Kit SECTOR	K150AYMU-1	K150AYMU-2	K150AYMU-4
Human/NHP IgG v2 Kit QuickPlex	K150AYMU-21	K150AYMU-22	K150AYMU-24
Human/NHP IgM v2 Kit SECTOR	K150AYPU-1	K150AYPU-2	K150AYPU-4
Human/NHP IgM v2 Kit QuickPlex	K150AYPU-21	K150AYPU-22	K150AYPU-24

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.

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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. IgG is commonly used as a research tool for humoral immunodeficiencies, autoimmune diseases, liver diseases, chronic inflammatory diseases, hematological 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 those 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

The assays in the MESO SCALE DISCOVERY® 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.

1. Capture antibodies are pre-coated to designated spots on the plate.
2. Analytes bridge the antibodies coated on the plate to the SULFO-TAG labeled detection antibodies.
3. An MSD® instrument applies voltage to the plate electrodes and measures the emitted light from the electrochemiluminescent SULFO-TAG™ label. The emitted light is proportional to the amount of analyte present in the sample.

1. IgA
3. IgG
10. IgM

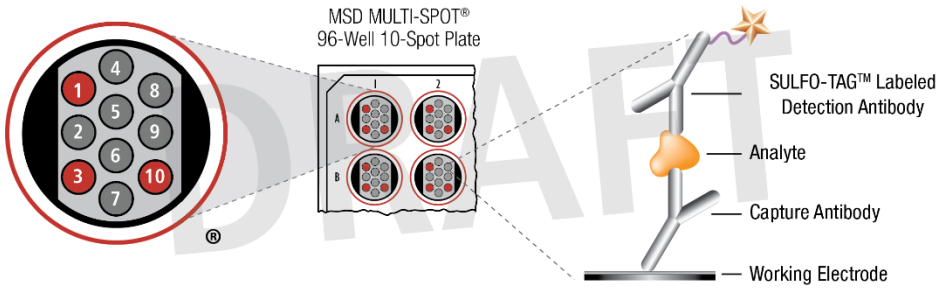


Figure 1. Spot diagram for the Isotyping Panel 1 (Human/NHP) Kit. Singleplex kits are also provided with the above plate, but with kit-specific detection antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the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

Reagents Supplied

Human/NHP Isotyping assays are available as a multiplex panel 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	
Human Isotyping Panel v2 96-well 10-Spot SECTOR Plate	2–8°C	N05770A-1	10-spot	1	5	25	96-well plate, foil sealed, with desiccant.
Human Isotyping Panel v2 96-well 10-Spot QuickPlex Plate	2–8°C	N0B770A-1	10-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.
		R50AA-3	1,000 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.
MSD GOLD™ Read Buffer B	RT	R60AM-1	50 mL	1 bottle	–	–	Buffer to catalyze the electro-chemiluminescence reaction.
		R60AM-2	90 mL	–	1 bottle	5 bottles	

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	D20AYM-2	75 µL	1			SULFO-TAG–conjugated antibody	Human/NHP IgG Kit
		D20AYM-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		

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 300–1,000 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

Read this product insert before use, and follow the best practices:

Reagent Preparation

Do Not Mix Lots	Do not mix components between boxes of multiplex panels. Lot information is provided in the lot-specific COC.
Thaw Reagents	Bring frozen diluents to room temperature in a 22–25 °C water bath. Thaw other reagents on wet ice and use them immediately. Mix well before use. Bring plates to room temperature before opening the packet.

Reagent Handling

Avoid Bubbles During Pipetting	Avoid bubbles at each stage of reagent addition because they can lead to variable results. This is especially important when adding read buffer (just prior to reading the plate).
Prepare in Polypropylene Microcentrifuge Tubes	Prepare standards and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution, and mix by vortexing after each dilution.
Avoid Prolonged Light Exposure	Avoid prolonged exposure of the detection reagent (stock or diluted) to light. During the plate incubation steps, plates do not need to be shielded from light (except for direct sunlight).

Plate Handling

Plate Shaking Guidelines	Plate shaking should be vigorous, with a rotary motion between 500 and 1,000 rpm for 96-well plates. Keep the shaking speed and shaker model consistent for long-term studies. Binding reactions may reach equilibrium sooner if shaken in the middle of this range (~700 rpm) or above.
Washing Fluid Removal	Tap the plate on a paper towel after washing to ensure the removal of residual fluid.
Some Assays are Temperature Sensitive	This kit was characterized at 20–26 °C. Assays run above or below that range may be negatively affected.

Plate Reading

Remove Plate Seal	Remove the plate seal before reading the plate in the instrument.
Do Not Shake Plate	Do not shake the plate after adding read buffer.
Room Temperature Read Buffer	Read buffer should be at room temperature (20-26 ºC) when adding it to the plate.
Timing of Plate Reads	Keep time intervals consistent between the addition of read buffer and reading the plate to improve interplate precision. Prepare an MSD instrument before adding 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 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.

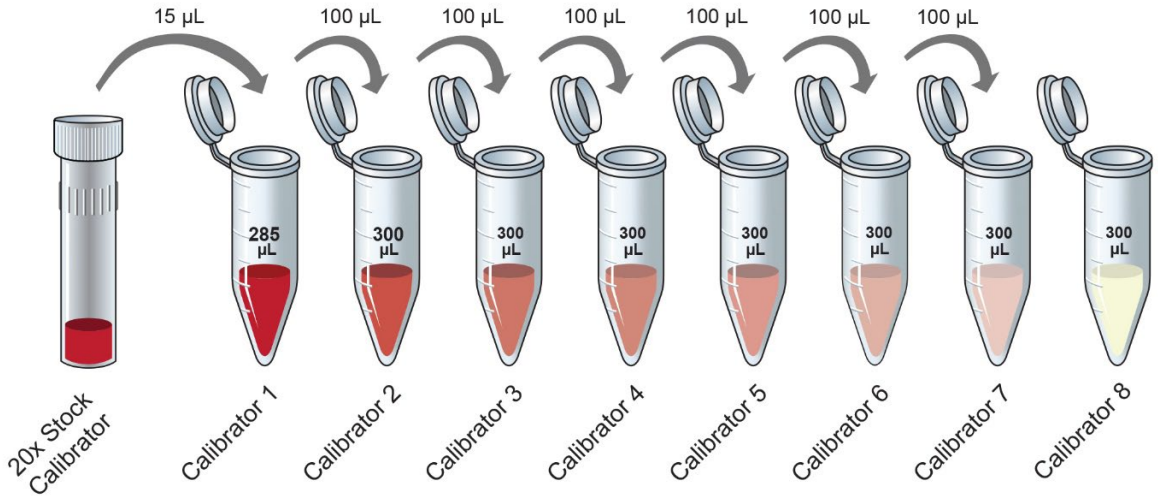


Figure 2. Dilution schema for preparation of Calibrator Standards.

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 100 µL of the highest calibrator to 300 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
- ❑ 2,820 µL of Diluent 100

Singleplex Kits

For 1 plate, add 60 µL of the supplied 50X detection antibody to 2,940 µL of Diluent 100.

Read Buffer

MSD provides MSD GOLD Read Buffer B ready to use; do not dilute.

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.

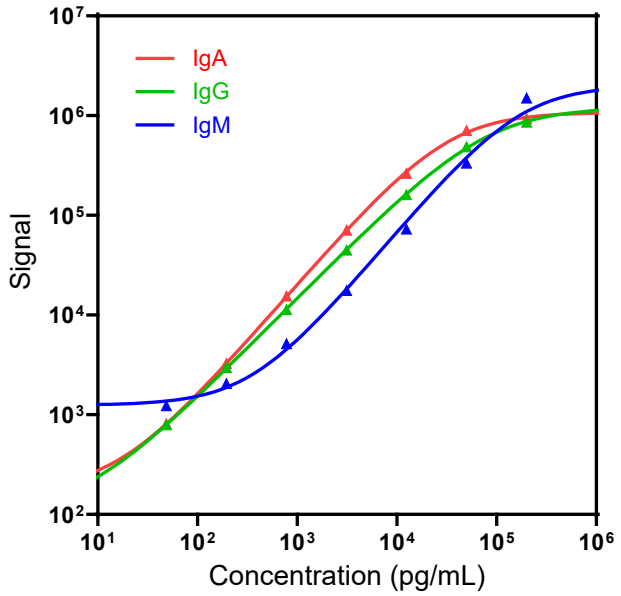
2. **Wash and Add Sample:** Wash the plate 3 times with at least 150 µL/well of PBS-T. Add 25 µL of diluted sample or calibrator per well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 2 hours.

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 MSD GOLD Read Buffer B to each well. Read the plate on the MSD instrument. No incubation in read buffer is required before reading the plate.

Typical Data

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



Sensitivity

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

	IgA	IgG	IgM
Average LLOD (pg/mL)	8.1	5.5	31

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.

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

		IgA		IgG		IgM	
Sample Type	Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Human Serum N=4	100,000	101	97–103	106	98–111	100	97–105
	500,000	102	101–103	94	93–98	102	98–105
	1,000,000	99	98–100	98	92–105	105	101–108
NHP Serum (Cynomolgus Monkey) N=4	100,000	77	73–81	98	86–117	92	90–96
	500,000	105	101–108	76	73–78	102	98–106
	1,000,000	109	105–114	57	46–67	103	102–105
NHP Serum (Rhesus Monkey) N=4	100,000	77	74–80	94	72–125	94	93–97
	500,000	109	103–114	141	101–170	102	101–103
	1,000,000	118	114–121	52	43–60	102	100–106

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)					
		Human Serum	Human Plasma	Cyno Serum	Cyno Plasma	Rhesus Serum	Rhesus Plasma
	N=	8	8	5	5	5	5
IgA	Median	1.9	2.0	1.9	1.2	1.8	1.1
	Range	1.5 -2.9	1.2 - 2.9	1.0 - 4.0	0.8 - 2.1	0.9 - 2.7	0.3 - 1.4
	% Detected	100	100	100	100	100	100
IgG	Median	5.4	5.3	0.3	0.6	0.7	0.2
	Range	3.9 - 8.3	3.9 - 8.3	0.2 - 1.1	0.4 - 0.8	0.4 - 2.2	0.1 - 0.4
	% Detected	100	100	100	100	100	100
IgM	Median	2.0	1.6	0.6	0.6	0.8	0.3
	Range	1.2 - 3.5	0.8 - 2.5	0.4 - 0.8	0.3 - 1.0	0.3 - 0.9	0.2 - 0.5
	% Detected	100	100	100	100	100	100

Specificity

To assess specificity, each assay in the panel was tested individually. Nonspecific binding was less than 1.0% 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

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Plate Diagram