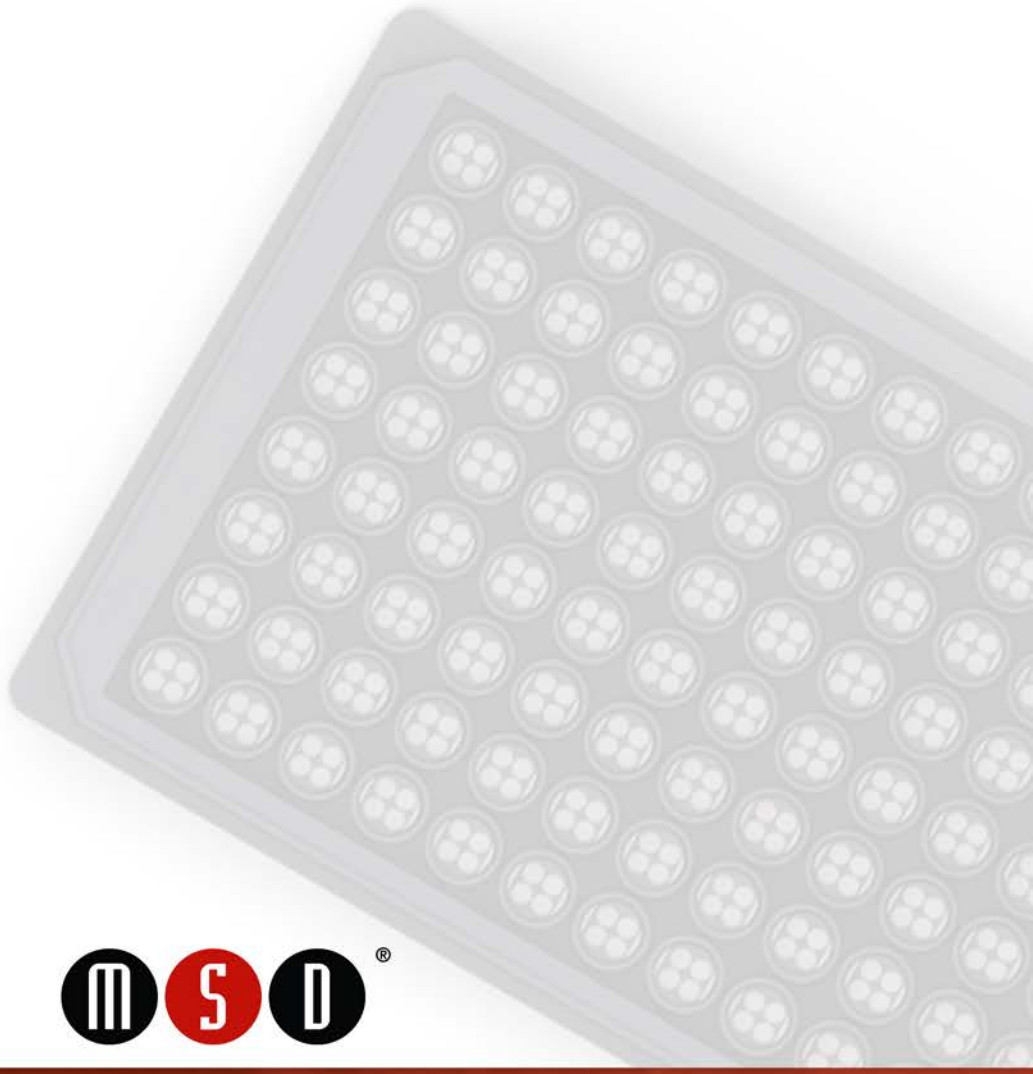
MSD® MULTI-SPOT Assay System

Liver Injury Panel 1 (rat) Assay Kit

1-Plate Kit	K15187D-1
5-Plate Kit	K15187D-2
25-Plate Kit	K15187D-4



MSD Toxicology Assays

Liver Injury Panel 1 (rat) Assay Kit

Arginase-1, αGST

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

MSD Customer Service

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Fax: 1-301-990-2776
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MSD Scientific Support

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Introduction

Arginase-1, also known as **Liver Type Arginase**, is the final enzyme of the urea cycle.[1] It is a ubiquitous cytosolic enzyme expressed at high levels in the liver of ureotelic animals and plays a key role in the disposal of excess nitrogen from amino acid and nucleotide metabolism. **Alpha Glutathione S-Transferase (αGST)** is a detoxification enzyme that catalyzes the conjugation of toxins to GST.[2] Both Arginase-1 and αGST have recently gained attention from the toxicology community due to their high levels in the liver, rapid release in the blood stream following liver injury, and short half-life in plasma.[3-6] These collective advantages may justify their use as alternative liver injury biomarkers over the conventional markers, alanine amino transferase (ALT), and aspartate amino transferase (AST). ALT and AST are widely distributed in a variety of tissues, and their elevated concentrations do not necessarily indicate hepatic disorder.[3,7] Arginase-1 and αGST offer an advantage in drug safety testing since they are found in the serum and plasma of both rats and humans; suggesting that they can be used to bridge pre-clinical and clinical studies.[7]

Principle of the Assay

MSD toxicology assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The assays are available in both singleplex and multiplex formats. In a singleplex assay, an antibody for a specific protein target is coated on one electrode (or "spot") per well. In a multiplex assay, an array of capture antibodies against different targets is patterned on distinct spots in the same well. The Liver Injury Panel 1 (rat) Assay is a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with capture antibodies for Arginase-1 and αGST on spatially distinct spots. The user adds the sample and a solution containing the detection antibodies—anti-Arginase-1 and anti-αGST conjugated with an electrochemiluminescent compound, 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 conjugated 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 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 intensity of emitted light to provide a quantitative measure of Arginase-1 and αGST present in the sample.

1. **Arginase-1**
2. *BSA blocked*
3. *BSA blocked*
4. **αGST**

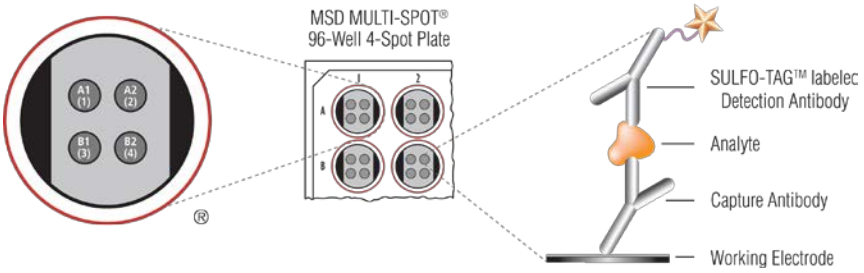


Figure 1. *Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing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

Reagents Supplied

Product Description	Storage	Quantity per Kit K15187D-1	K15187D-2	K15187D-4
MULTI-SPOT 96-Well 4-Spot Liver Injury Panel 1 (rat) Plate N45187A-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-rat Arginase-1 Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
SULFO-TAG Anti-rat αGST Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Liver Injury Panel 1 (rat) Calibrator Blend (20X)	≤-70°C	1 vial (20 µL)	5 vials (20 µL ea)	25 vials (20 µL ea)
Diluent 100 R50AA-4 (50 mL), R50AA-2 (200 mL)	2–8°C	1 bottle (50 mL)	1 bottle (200 mL)	5 bottles (200 mL ea)
Blocker D-R[2] (10%)	≤-10°C	1 vial (0.05 mL)	2 vials (0.2 mL ea)	10 vials (0.2 mL ea)
Blocker A Kit R93AA-2 (250 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	5 bottles (250 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

Additional Materials and Equipment

- ❑ Appropriately sized 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 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
- ❑ Deionized water

[1] SULFO-TAG-conjugated detection antibodies should be stored in the dark.
[2] Upon first thaw, separate Blocker D-R into aliquots appropriate to the size of your assay needs. The blocker can tolerate up to two freeze-thaw cycles.

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.

Reagent Preparation

Bring all reagents to room temperature. Thaw the stock calibrator blend on ice.

Prepare Blocker A Solution

Follow the Blocker A instructions included in the kit.

Prepare Calibrator Dilutions

Calibrators for the Liver Injury Panel 1 (rat) Assay are supplied at 20-fold higher concentration than the recommended highest calibrator. For each assay, an 8-point standard curve is recommended with 3-fold serial dilution steps and a zero calibrator. The calibrators are supplied as a blend. The stock calibrator blend should be thawed and kept on ice and then should be added into diluent at room temperature to make the standard curve solutions. For the actual concentration of the calibrator, refer to the certificate of analysis (COA) supplied with the kit. A copy of the kit-specific COA can also be found at www.mesoscale.com

To prepare an 8-point standard curve for up to 3 replicates:

1) Prepare the highest calibrator (standard 1) by adding 10 µL of the calibrator stock vial to 190 µL of Diluent 100. Mix well.
2) Prepare the next calibrator (standard 2) by transferring 80 µL of the diluted calibrator to 160 µL of Diluent 100. Mix well. Repeat 3-fold serial dilutions 5 additional times to generate 7 calibrators (standards 3 through 7).
3) The recommended 8th standard is Diluent 100 (i.e. zero calibrator).

Calibrators should be prepared at room temperature no more than 30 minutes before use.

Dilution of Samples

Some rat samples should be diluted prior to the assay in order to get the analyte levels into the detection range. A 5-fold dilution of samples into Diluent 100 is recommended for this multiplex panel. Depending on the sample set under investigation, higher or lower dilution factors may be necessary.

Prepare Detection Antibody Solution

Each detection antibody is provided as a 50X stock solution. The final concentration of the working detection antibody solution should be at 1X.

In a 15 mL tube combine (per plate):

- ❑ 60 µL of 50X SULFO-TAG Anti-rat Arginase-1 Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-rat αGST Antibody
- ❑ 30 µL of 10% Blocker D-R
- ❑ 2850 µL of Diluent 100

Prepare Read Buffer

The Read Buffer T (4X) should be diluted 4-fold in deionized water to make a final concentration of 1X Read Buffer T. Add 5 mL of Read Buffer T (4X) to 15 mL of deionized water for each plate.

Prepare MSD Plate

This plate has been pre-coated with antibodies for the analytes 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.

Assay Protocol

Notes

1. **Addition of Blocker A Solution:** Dispense 150 µL of Blocker A solution into each well of the MSD plate. Seal the plate with an adhesive plate seal, and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

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

2. **Wash and Addition of the Sample or Calibrator:** Wash the plate 3 times with 300 µL/well of PBS-T. First, dispense 25 µL of Diluent 100 into each well of the MSD plate. Then add 25 µL of diluted 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 3 times with 300 µL/well of PBS-T. Dispense 25 µL of 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 3 times with 300 µL/well of PBS-T. Add 150 µL of 1X Read Buffer T to each well of the MSD plate. Analyze the plate on an MSD instrument. Read the plate within 10 minutes of read buffer addition.

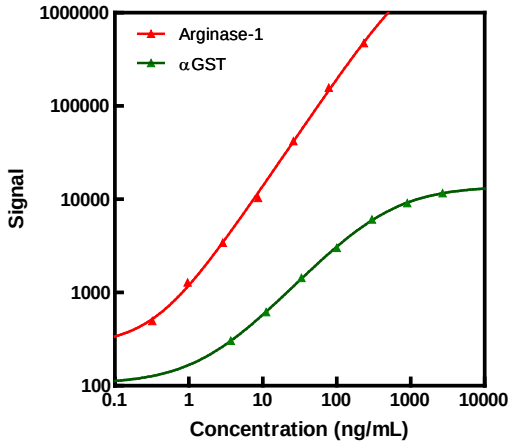
Bubbles in the fluid will interfere with reliable reading of MULTI-SPOT plate. Use reverse pipetting techniques to ensure bubbles are not created when dispensing the read buffer.

Analysis of Results

The calibrators should be run minimally 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 quantification 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 Data

The following standard curves are 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 quantification of unknown samples.



Arginase-1		
Conc. (ng/mL)	Average Signal	%CV
0	99	10.0
0.32	496	6.1
0.96	1280	10.4
2.9	3416	1.3
8.7	10 352	1.4
26	41 933	5.0
78	157 125	4.7
234	473 734	6.5

αGST		
Conc. (ng/mL)	Average Signal	%CV
0	127	7.3
3.7	304	9.0
11	617	7.2
33	1432	2.8
100	3012	4.6
300	6072	3.0
901	9114	2.1
2703	11 623	2.7

Sensitivity

The lower limit of detection (LLOD) is the calculated concentration of the signal that is 2.5 standard deviations over the blank (zero calibrator).

	Arginase-1 (ng/mL)	αGST (ng/mL)
LLOD	0.032	0.42

Precision

Control samples for each analyte were measured on each plate. Controls were made by spiking high, mid, and low (no spike) levels of calibrators into rat heparin plasma.

The controls were run in duplicate on each of 12 plates run across multiple days (n=10).

The concentrations shown below have not been corrected for dilution.

Average intra-plate %CV is the average %CV of the control replicates within an individual plate.

Inter-plate %CV is the variability of controls across 12 plates over 10 days.

	Control	Plates	Average Conc. (ng/mL)	Average Intra-plate %CV	Inter-plate %CV
Arginase-1	High	12	97	4.0	9.3
	Mid	12	12	6.7	11.3
	Low	12	0.87	4.5	11.0
αGST	High	12	823	11.4	10.3
	Mid	12	140	4.1	9.7
	Low	12	11	5.2	10.4

Spike Recovery

Normal serum, EDTA plasma, and heparin plasma were spiked with the calibrators at multiple levels throughout the range of the assay. The samples were diluted 5-fold and then spiked with calibrator at the levels indicated in the table below.

% Recovery = measured / expected x 100

	Arginase-1				αGST			
Sample	Spike Conc. (ng/mL)	Measured Conc. (ng/mL)	Measured Conc. %CV	% Recovery	Spike Conc. (ng/mL)	Measured Conc. (ng/mL)	Measured Conc. %CV	% Recovery
Serum 1	0	0.85	0.2		0	14	4.2	
	2.9	4.1	3.8	108	34	49	6.4	103
	12	12	1.6	98	135	140	3.9	94
	23	25	1.1	102	270	299	3.1	105
	47	52	7.2	108	541	519	13.0	94
	94	120	4.0	127	1081	945	1.9	86
Serum 2	0	1.1	8.8		0	27	1.4	
	2.9	3.6	0.1	90	34	62	0.9	101
	12	12	1.6	91	135	150	1.8	92
	23	23	0.1	92	270	281	0.7	95
	47	44	0.5	92	541	530	0.3	93
	94	98	1.5	104	1081	914	5.5	82
EDTA Plasma 1	0	1.1	12.8		0	6.7	0.9	
	2.9	3.5	9.1	86	34	33	3.8	83
	12	10	3.3	78	135	94	6.1	66
	23	18	8.8	73	270	171	9.7	62
	47	37	18.6	77	541	298	8.0	54
	94	71	18.7	75	1081	478	6.2	44
EDTA Plasma 2	0	0.51	4.0		0	4.8	5.6	
	2.9	2.6	6.6	74	34	29	10.1	76
	12	10	1.6	84	135	103	0.8	74
	23	20	13.3	83	270	181	2.6	66
	47	38	5.6	80	541	322	8.4	59
	94	72	6.2	77	1081	516	1.9	48
Heparin Plasma 1	0	0.46	5.3		0	9.5	0.7	
	2.9	3.4	1.2	100	34	41	5.4	94
	12	11	5.2	90	135	113	0.4	78
	23	20	0.4	85	270	223	6.0	80
	47	47	2.5	99	541	433	4.0	79
	94	103	0.2	109	1081	850	1.0	78
Heparin Plasma 2	0	0.76	6.9		0	12	5.8	
	2.9	3.6	3.3	97	34	40	0.3	88
	12	11	1.9	91	135	106	3.1	72
	23	24	9.9	99	270	209	9.1	74
	47	53	2.6	111	541	410	1.8	74
	94	117	4.2	124	1081	701	4.7	64

Linearity

To assess linearity, serum, EDTA plasma, and heparin plasma samples were diluted 2.5-fold, 5-fold, 10-fold, and 20-fold prior to testing. 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 measured from the previous dilution (expected).

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

		Arginase-1			αGST		
Sample	Fold Dilution	Conc. (ng/mL)	Conc. %CV	% Recovery	Conc. (ng/mL)	Conc. %CV	% Recovery
Serum	2.5	4.8	9.8		129	2.1	
	5	5.8	13.4	120	143	5.4	111
	10	5.9	5.2	102	140	6.9	98
	20	<LLOD	-	-	121	10.6	86
EDTA Plasma	2.5	4.5	0.4		32	2.4	
	5	4.9	14.3	108	33	3.3	102
	10	5.7	4.2	116	33	0.4	103
	20	<LLOD	-	-	32	2.9	96
Heparin Plasma	2.5	2.1	8.3		48	6.9	
	5	2.5	7.7	121	55	6.0	115
	10	2.6	2.7	102	<LLOD	-	-
	20	<LLOD	-	-	<LLOD	-	-

Specificity

Specificity of the Detection Antibodies

In order to assess specificity of the detection antibodies, the Liver Injury Panel 1 (rat) Assay was run with blended calibrators diluted to standard 2, and individual detection antibodies. The % cross-reactivity for each individual detection antibody is shown below.

	Blended Calibrator and Single Detection Antibody % Cross-Reactivity	
Spot	Arginase-1	αGST
Arginase-1	100	10
αGST	0.23	100

Species Cross-Reactivity for Arginase-1

Liver Injury Panel 1 (rat) Assay was run with recombinant human Arginase-1. The table below shows Arginase-1 signal from recombinant human Arginase-1. Recombinant human Arginase-1 is recognized by our current Arginase-1 antibody pair, but with about 20-fold less sensitivity compared to signals from rat Arginase-1.

	Recombinant Human Arginase-1	
Conc (ng/mL)	Average Signal	%CV
0	68	12.5
1.5	132	10.7
4.6	194	25.5
13.7	590	3.1
41.2	2074	3.5
123	7234	0.6
370	30 453	3.9
1111	81 736	1.6
3333	164 340	1.9
10 000	255 711	2.4

Samples

Serum, EDTA plasma, and heparin plasma samples collected from normal Sprague-Dawley rats were tested at 5-fold dilution on the Liver Injury Panel 1 (rat) Assay. Shown below are the median and range of concentrations for each sample set. Concentrations have been corrected for sample dilution.

Sample	Statistic	Arginase-1	αGST
Serum	Median (ng/mL)	22	642
	Range (ng/mL)	3.6-109	44-1826
	N	8	8
EDTA Plasma	Median (ng/mL)	4.7	52
	Range (ng/mL)	1.5-12	25-310
	N	8	7
Heparin Plasma	Median (ng/mL)	3.2	46
	Range (ng/mL)	2.2-7.9	21-176
	N	8	8

Assay Components

Calibrators

Liver homogenate from rat is used to make the Liver Injury Panel 1 (rat) Calibrator Blend. The liver homogenate contains both analytes. Rat liver homogenate is calibrated against internal controls, and then diluted to make the Liver Injury Panel 1 (rat) Calibrator Blend. Because of variability between batches of the liver homogenate, the concentration values of the calibrators are lot-specific.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
Arginase-1	Mouse Monoclonal	Mouse Monoclonal
αGST	Rabbit Polyclonal	Rabbit Polyclonal

References

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Summary Protocol

MSD 96-well MULTI-SPOT Liver Injury Panel 1 (rat) Assay Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Liver Injury Panel 1 (rat) Assay.

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature, and thaw the calibrator on ice.
- ❑ Prepare an 8-point standard curve using the supplied calibrators:
  - The calibrator blend should be diluted in Diluent 100.
  - Dilute the stock calibrator blend 20-fold in Diluent 100. Then perform a series of 3-fold dilution steps and prepare a zero calibrator blank.
  - Dilute samples 5-fold into Diluent 100 prior to addition to the plate.
- ❑ Prepare detection antibody solution by diluting the 50X detection antibodies to a 1X final concentration of each antibody. The detection antibodies should be diluted in a final volume of 3.0 mL Diluent 100 + 0.1% Blocker D-R per plate.
- ❑ Prepare 20 mL of 1X Read Buffer T by diluting 4X Read Buffer T with deionized water.

Step 1 : Add Blocker A Solution

- ❑ Dispense 150 µL/well of Blocker A Solution.
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 2 : Wash Add Sample or Calibrator

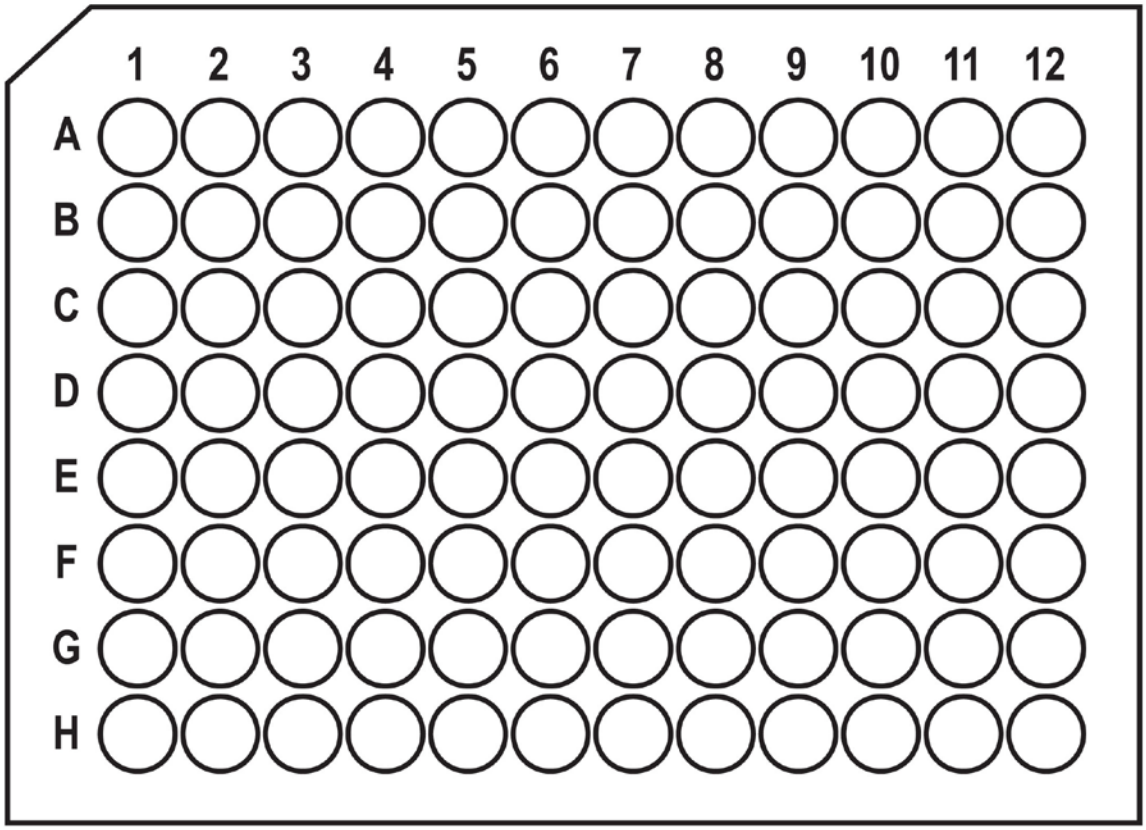
- ❑ Wash plate 3 times with 300 µL/well of PBS-T.
- ❑ Dispense 25 µL/well of Diluent 100 followed by 25 µL of calibrator or diluted sample.
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 3 : Wash and Add Detection Antibody Solution

- ❑ Wash plate 3 times with 300 µL/well of PBS-T.
- ❑ Dispense 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 plate 3 times with 300 µL/well of PBS-T.
- ❑ Dispense 150 µL/well of 1X Read Buffer T.
- ❑ Analyze plate on an MSD instrument.
- ❑ Read the plate within 10 minutes of read buffer addition.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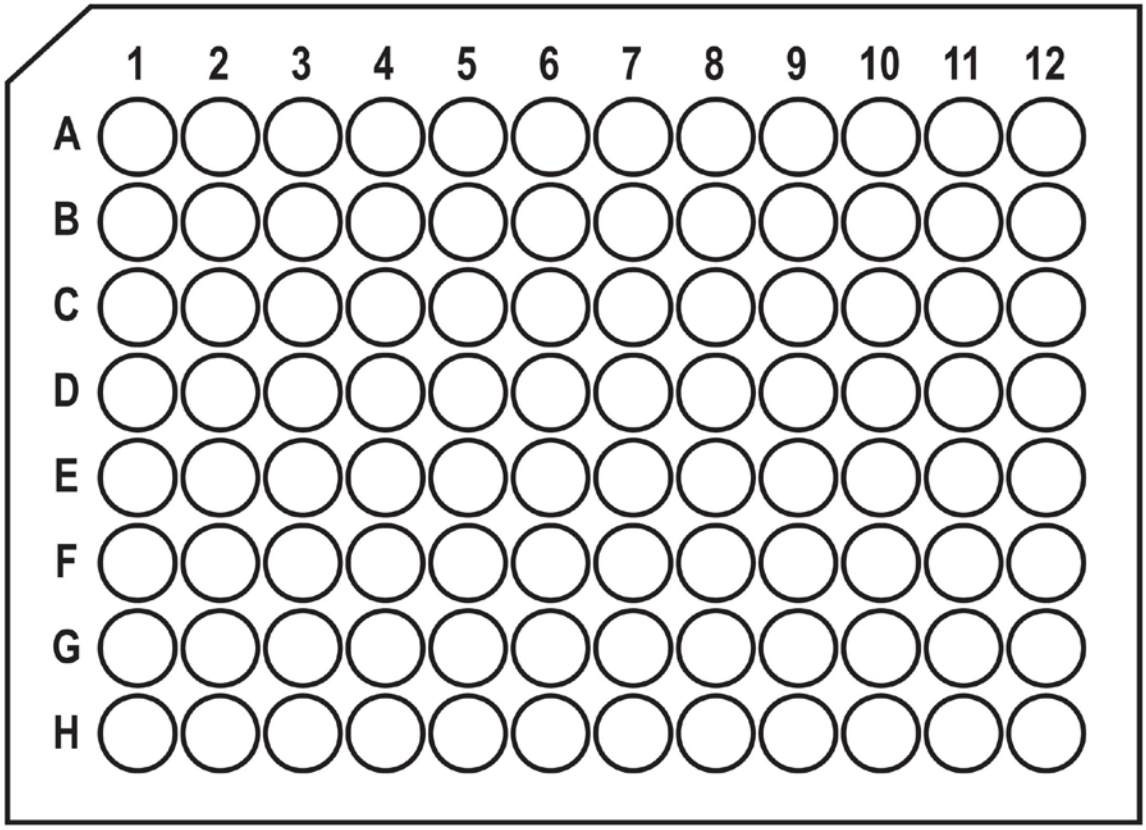
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