

# MSD<sup>®</sup> MULTI-SPOT Assay System

## Liver Injury Panel 1 (rat) Assay Kit

1-Plate Kit  
5-Plate Kit  
25-Plate Kit

K15187D-1  
K15187D-2  
K15187D-4



# MSD Toxicology Assays

## Liver Injury Panel 1 (rat) Assay Kit

Arginase-1,  $\alpha$ GST

*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<sup>®</sup>**

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# MSD Advantage

MESO SCALE DISCOVERY'S unique spot patterns are a hallmark of our MULTI-ARRAY<sup>®</sup> technology, which enables the measurement of biomarkers utilizing the next generation of electrochemiluminescent detection. In an MSD assay, specific capture antibodies for the analytes are coated in arrays in each well of a 96-well carbon electrode plate surface. The detection system uses patented SULFO-TAG<sup>™</sup> labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of the MULTI-ARRAY and MULTI-SPOT<sup>®</sup> plates. The electrical stimulation is decoupled from the output signal, which is light, to generate assays with minimal background. MSD labels can be conveniently conjugated to biological molecules, are stable, and are non-radioactive. Additionally, only labels near the electrode surface are detected, enabling non-washed assays.

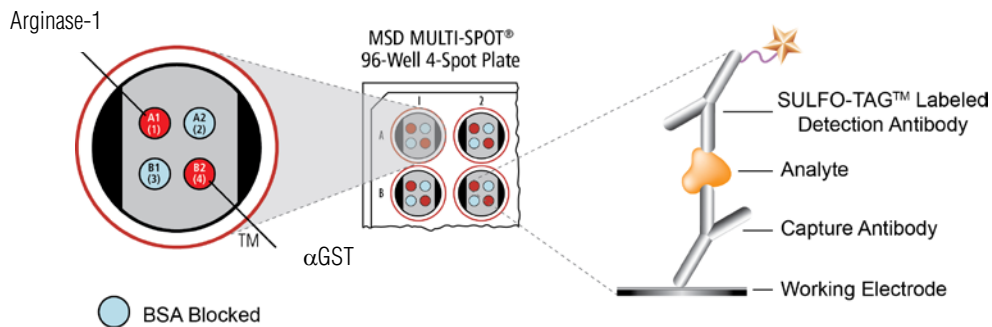
One of the advantages of MSD assays is the minimal sample volume required as compared to a traditional ELISA, which is also limited by its inability to measure more than a single analyte. With an MSD assay, up to ten different biomarkers can be analyzed simultaneously using as little as 10-25  $\mu$ L of sample. These assays have high sensitivity, up to five logs of linear dynamic range, and excellent performance in complex biological matrices. Combined, these advantages enable the measurement of native levels of biomarkers in normal and diseased samples without multiple dilutions. Further, the simple and rapid protocols of MSD assays provide a powerful tool to generate reproducible and reliable results. The MSD product line offers a diverse menu of assay kits for profiling biomarkers, cell signaling pathways, and other applications, as well as a variety of plates and reagents for assay development.

## Introduction

**Arginase-1**, also known as **Liver Type Arginase**, is the final enzyme of the urea cycle.<sup>1</sup> 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 ( $\alpha$ GST)** is a detoxification enzyme that catalyzes the conjugation of toxins to GST.<sup>2</sup> Both Arginase-1 and  $\alpha$ GST 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.<sup>3-6</sup> 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.<sup>3,7</sup> Arginase-1 and  $\alpha$ GST 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.<sup>7</sup>

# 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  $\alpha$ GST on spatially distinct spots. The user adds the sample and a solution containing the detection antibodies—anti-Arginase-1 and anti- $\alpha$ GST 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 SECTOR<sup>®</sup> Imager for analysis. Inside the SECTOR Imager, 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  $\alpha$ GST present in the sample.



**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 <sup>1</sup> (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
SULFO-TAG Anti-rat αGST Antibody <sup>1</sup> (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 <sup>2</sup> (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)

## Required Materials and Equipment - not supplied

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

<sup>1</sup> Some SULFO-TAG conjugated detection antibodies may be light-sensitive, so they should be stored in the dark.

<sup>2</sup> 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

Safe laboratory practices and personal protective equipment such as gloves, safety glasses, and lab coats should be used at all times during the handling of all kit components. All hazardous samples should be handled and disposed of properly, in accordance with local, state, and federal guidelines.

## Reagent Preparation

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

### Prepare Blocker A Solution

Follow instructions included with the Blocker A Kit.

### Prepare Calibrator and Control Solutions

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 (C of A) supplied with the kit. A copy of the kit-specific C of A can also be found at [www.mesoscale.com](http://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  $\mu\text{L}$  of the calibrator stock vial to 190  $\mu\text{L}$  of Diluent 100. Mix well.
- 2) Prepare the next calibrator (standard 2) by transferring 80  $\mu\text{L}$  of the diluted calibrator to 160  $\mu\text{L}$  of Diluent 100. Mix well. Repeat 3-fold serial dilutions 5 additional times to generate 7 calibrators (standards 3 through 7).
- 3) The recommended 8<sup>th</sup> 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  $\mu$ L of 50X SULFO-TAG Anti-rat Arginase-1 Antibody
- 60  $\mu$ L of 50X SULFO-TAG Anti-rat  $\alpha$ GST Antibody
- 30  $\mu$ L of 10% Blocker D-R
- 2850  $\mu$ L 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  $\mu\text{L}$  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.
2. **Wash and Addition of the Sample or Calibrator:** Wash the plate 3 times with 300  $\mu\text{L}$ /well of PBS-T. First dispense 25  $\mu\text{L}$  of Diluent 100 into each well of the MSD plate. Then add 25  $\mu\text{L}$  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  $\mu\text{L}$ /well of PBS-T. Dispense 25  $\mu\text{L}$  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  $\mu\text{L}$ /well of PBS-T. Add 150  $\mu\text{L}$  of 1X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. Read the plate within 10 minutes of read buffer addition.

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

*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

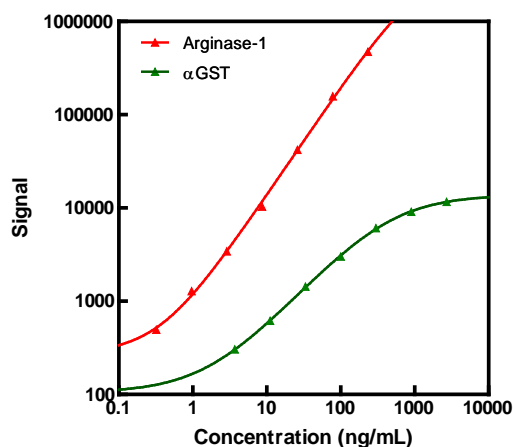
The calibrators should be run minimally in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have a wide dynamic range (3 - 4 logs) which allows accurate quantification in many samples without the need for dilution. The MSD DISCOVERY WORKBENCH<sup>®</sup> 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 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.

Some variation in the concentration of the highest calibrator is permissible between kit lots. Below is a table that details the acceptable range of the highest calibrator concentration. For each individual kit lot, the calibrator concentrations are shown in the C of A.

	Highest Calibrator Concentration	
	Target	Acceptable range
Arginase-1 (ng/mL)	234	164-304
αGST (ng/mL)	2703	1892-3514



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	10352	1.4
26	41933	5.0
78	157125	4.7
234	473734	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	11623	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

Sample	Arginase-1				αGST			
	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).

$$\% \text{ Recovery} = (\text{measured} \times \text{dilution factor}) / \text{expected} \times 100$$

Sample	Fold Dilution	Arginase-1			αGST		
		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.

Spot	Blended Calibrator and Single Detection Antibody % Cross-Reactivity	
	Arginase-1	$\alpha$ GST
Arginase-1	100	10
$\alpha$ GST	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.

Conc (ng/mL)	Recombinant Human Arginase-1	
	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	30453	3.9
1111	81736	1.6
3333	164340	1.9
10000	255711	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	$\alpha$ GST
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*

Analyte	Source Species	
	MSD Capture Antibody	MSD Detection Antibody
Arginase-1	Mouse Monoclonal	Mouse Monoclonal
$\alpha$ GST	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.

#### Step 1 : 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 by 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 2 : Add Blocker A Solution

Dispense 150  $\mu$ L/well of Blocker A Solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

#### Step 3 : Wash Add Sample or Calibrator

Wash plate 3 times with 300  $\mu$ L/well of PBS-T.

Dispense 25  $\mu$ L/well of Diluent 100 followed by 25  $\mu$ L of calibrator or diluted sample.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

#### Step 4 : Wash and Add Detection Antibody Solution

Wash plate 3 times with 300  $\mu$ L/well of PBS-T.

Dispense 25  $\mu$ L/well of 1X detection antibody solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

#### Step 5 : Wash and Read Plate

Wash plate 3 times with 300  $\mu$ L/well of PBS-T.

Dispense 150  $\mu$ L/well of 1X Read Buffer T.

Analyze plate on SECTOR Imager.

Read the plate within 10 minutes of read buffer addition.



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D	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
F	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
G	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
H	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

	1	2	3	4	5	6	7	8	9	10	11	12
A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
B	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
C	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
D	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
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G	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
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