Novel Liver Injury Biomarkers: Arginase-1 and αGST

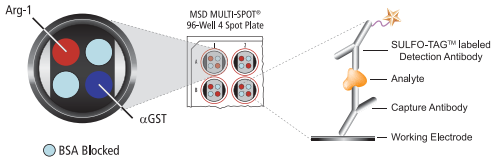
The measurement of protein biomarkers as indicators of drug toxicity shows promise to improve drug safety and accelerate the development timeline. Effective safety assessment depends on robust assays that are sensitive enough to detect minor variations from the native protein levels in complex sample matrices and perform consistently within and across studies.

MESO SCALE DISCOVERY® (MSD) has developed the MULTI-SPOT® Liver Injury Panel 1 (rat) assay to measure Arginase-1 and αGST in serum and plasma samples. These emerging biomarkers serve as indicators of various types and stages of liver injury to achieve a comprehensive view of drug-induced hepatoxicity. Experiments to characterize the assay verify that this panel offers the standard advantages of MSD® assays: high sensitivity, reproducibility, reduced sample volume, wide dynamic range (allowing endogenous and elevated levels to be measured at a single sample preparation), and improved assay throughput over other methods.

Description of Markers

Arginase-1 (Arg-1) is a ubiquitous cytosolic enzyme expressed at high levels in the liver of ureotelic animals. It plays a key role in the disposal of excess nitrogen from amino acid and nucleotide metabolism. Arg-1 has recently gained importance as one of the markers for assessment of liver damage, particularly in the acute phase of hepatocellular injury.

Alpha Glutathione S-Transferase (**αGST**), a detoxification enzyme, catalyzes the conjugation of toxins to GST. It is a specific and sensitive marker of acute hepatocyte injury and recovery.

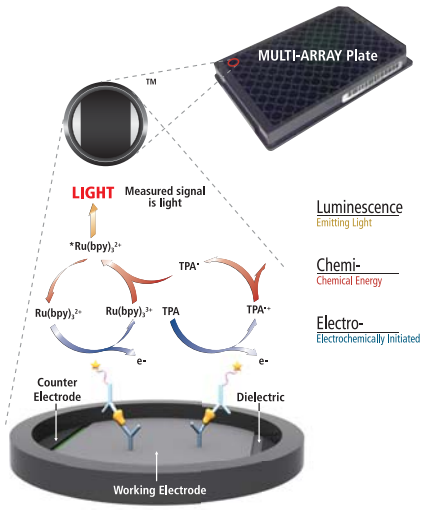


Protocol:

1 Add 150 µL blocking solution, incubate for 1 hour at RT.
2 Wash with PBS-T. Add 25 µL Diluent 100 and then 25 µL standard or sample. Incubate for 2 hours at RT.
3 Wash with PBS-T. Add 25 µL of detection antibody. Incubate for 2 hours at RT.
4 Wash with PBS-T. Add 150 µL of Read Buffer T and then read on a SECTOR® Imager.

The MSD Platform

MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates.

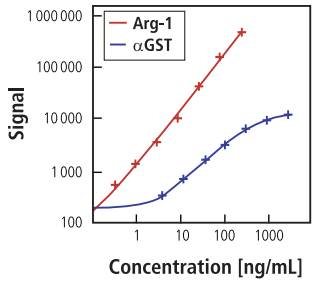


Electrochemiluminescence Features:

- Minimal non-specific backgrounds and strong signal responses to analyte yield high signal to background ratios
- The stimulation mechanism (electricity) is decoupled from the response (light signal)
- Proximity assay - only labels bound near the electrode surface are excited, enabling non-washed assays
- Flexibility - labels are stable, non-radioactive, and directly conjugated to biological molecules
- Emission at ~620 nm - eliminating problems with color quenching
- Signal amplification - multiple rounds of excitation and emission of each label enhance light levels and improve sensitivity
- Carbon electrode surface has 10X greater binding capacity than polystyrene well
- Surface coatings can be customized

Standard Curve

The following standard curves show the dynamic range of the assays in the Liver Injury Panel 1(rat). The actual signals may vary, thus a standard curve should be run on each plate for the best quantitation of analytes in samples.



Arg-1		
Concentration (ng/mL)	Average Signal	% CV
0	99	10.0
0.321	496	6.1
0.963	1280	10.4
2.89	3416	1.3
8.67	10352	1.4
26.0	41933	5.0
78.0	157125	4.7
234	473734	6.5

αGST		
Concentration (ng/mL)	Average Signal	% CV
0	127	7.3
3.71	304	9.0
11.1	617	7.2
33.4	1432	2.8
100	3012	4.6
300	6072	3.0
901	9114	2.1
2703	11623	2.7

	Arg-1	αGST
LLOD (ng/mL)	0.0320	0.421

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

Precision: Multi-Day Study

Control samples of high, mid, and low levels of analyte were prepared in rat heparin plasma. These control samples were used to assess intra- and inter-plate precision.

	Control	Plates	Average Measured Conc. (ng/mL)	Average Intra-plate % CV	Inter-plate % CV
Arg-1	High	4	99.9	3.2	3.8
	Mid	4	12.3	3.6	11.1
	Low	4	0.895	4.7	11.5
αGST	High	4	842	2.8	8.6
	Mid	4	140	2.4	7.6
	Low	4	9.51	9.8	15.4

Spike Recovery

Two different pools of rat heparin plasma samples were diluted 5-fold and then spiked with calibrators at multiple levels throughout the range of the assay.
Results of spike recovery may vary based upon differences between individual plasma samples.
% Recovery = measured / expected x 100

	Arg-1			
	Spike Conc. (ng/mL)	Measured Conc. (ng/mL)	Measured Conc. % CV	% Recovery
Heparin Plasma 1	93.6	101	1.6	107
	46.8	48.8	4.1	103
	15.6	16.4	6.8	102
	0	0.461	5.8	
Heparin Plasma 2	93.6	96.5	3.0	103
	46.8	51.4	0.8	109
	15.6	14.7	2.5	92
	0	0.329	6.2	

	αGST			
	Spike Conc. (ng/mL)	Measured Conc. (ng/mL)	Measured Conc. % CV	% Recovery
Heparin Plasma 1	1081	1018	10.9	94
	541	547	3.2	100
	180	184	2.4	99
	0	6.12	13.9	
Heparin Plasma 2	1081	941	1.9	87
	541	495	1.5	91
	180	173	0.3	93
	0	5.17	5.7	

Dilution Linearity

Two different pools of rat heparin plasma samples were serially diluted four times with the assay diluents. The concentrations have been corrected for dilution (concentration = measured x dilution factor). Percent recovery is calculated as the measured concentration divided by the concentration of the previous dilution (expected).
% Recovery = (measured x dilution factor) / expected x 100

	Arg-1			
	Fold Dilution	Conc. (ng/mL)	Conc. % CV	% Recovery
Heparin Plasma 1	2.5	4.40	0.4	
	5	4.66	6.3	106
	10	4.70	0.2	101
	20	5.50	5.1	117
Heparin Plasma 2	2.5	2.86	1.1	
	5	3.16	0.7	110
	10	3.49	1.6	110
	20	4.12	11.7	118

	αGST			
	Fold Dilution	Conc. (ng/mL)	Conc. % CV	% Recovery
Heparin Plasma 1	2.5	55.4	2.3	
	5	66.1	2.3	119
	10	72.5	7.9	110
	20	71.2	8.0	98
Heparin Plasma 2	2.5	45.6	5.1	
	5	55.2	1.4	121
	10	59.9	1.2	108
	20	62.2	10.3	104

Specificity of the Arg-1

Specificity of the Arg-1 assay on the Liver Injury Panel 1 (rat) was confirmed by testing homogenates from liver (specific for Arg-1), kidney (specific for Arg-2), and quadriceps (skeletal muscle) to represent a non-specific tissue.

The Arg-1 assay was run with these three tissue homogenates (diluted 50- and 500-fold). The table shows the average signals for each homogenate.

Approximately 3000- and 27000-fold higher signal was generated from liver compared to kidney and quadriceps respectively. This data confirms the specificity of the Arg-1 assay on the Liver Injury Panel 1 (rat).

	Fold Dilution	Average Signal	% CV
Liver Homogenate (rat)	50	974886	2.5
	500	107316	0.2
Kidney Homogenate (rat)	50	333	9.3
	500	123	8.0
Quadriceps Homogenate (rat)	50	36	10.0
	500	33	25.7
Assay Diluent	N/A	98	11.5

Specificity of the detection antibodies

The Liver Injury Panel 1 (rat) was run with blended calibrators diluted to 78 ng/mL Arg-1 and 901 ng/mL αGST and individual detection antibodies.

The table shows the % cross-reactivity for each individual detection antibody.

	Blended Calibrator and Single Detection Antibody % Cross-Reactivity	
Spot	Arg-1	αGST
Arg-1	100	10
αGST	0.2	100

Samples

The following samples were assessed with the Liver Injury Panel 1 (rat) assay:

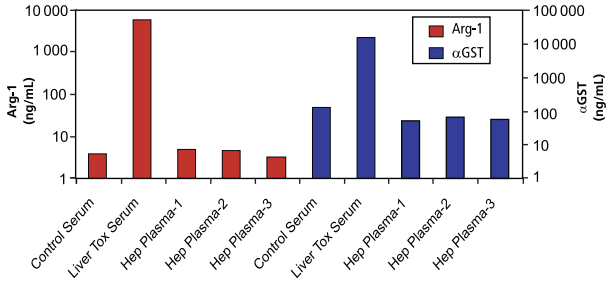
- Rat serum (pooled normal samples)
- Rat serum (pooled samples from animals with liver toxicity)
- Rat heparin plasma (3 pooled normal samples)

These samples demonstrate a good correlation between Arg-1 and αGST.

There was approximately 1600- and 100-fold signal difference observed between liver tox and control serum for Arg-1 and αGST respectively.

	Arg-1 (ng/mL)	Conc. % CV	αGST (ng/mL)	Conc. % CV
Control Serum	3.78	11.5	139	5.6
Liver Tox Serum	6105	10.9	15141	1.2
Heparin Plasma 1	5.21	3.3	53.3	1.4
Heparin Plasma 2	4.66	6.3	66.1	2.3
Heparin Plasma 3	3.16	0.671	55.2	5.2

Endogenous Arg-1 and αGST in Serum and Plasma



Conclusions

MSD's MULTI-SPOT Liver injury Panel 1 (rat) assay simultaneously detects and quantifies native Arg-1 and αGST in plasma and serum samples. Both analytes can be measured accurately in plasma and serum when the samples are diluted at least 5-fold. Through the monitoring of Arg-1 and αGST, the assay can be used to assess drug-related liver injury.