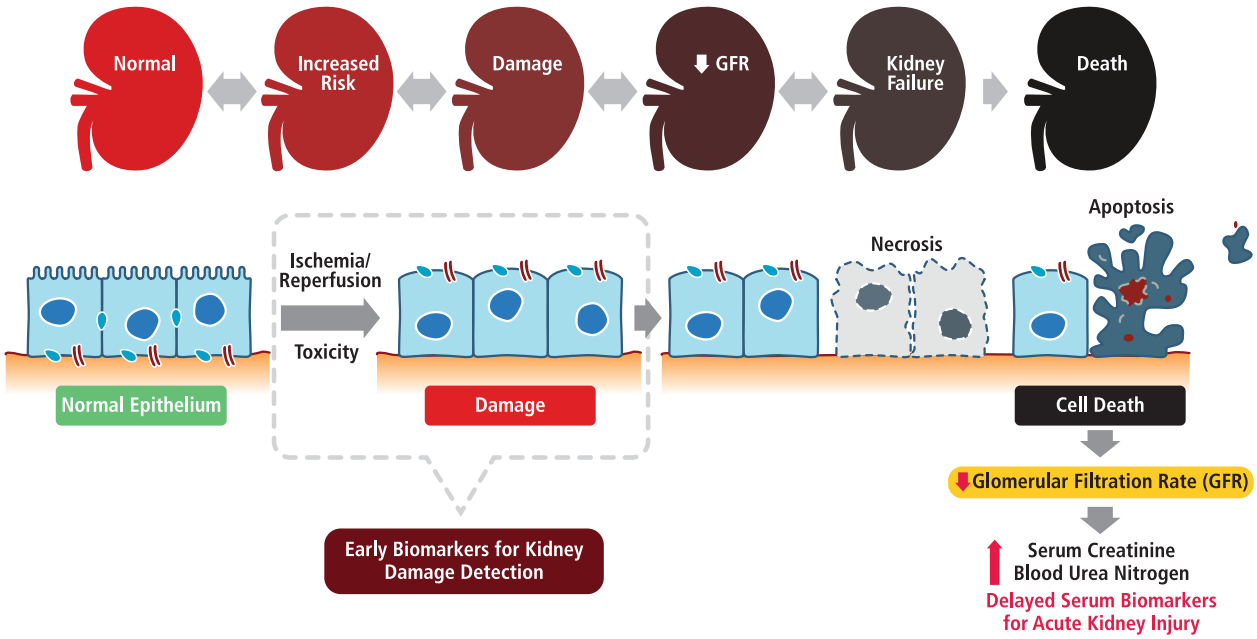
Development of Human Kidney Injury Biomarker Assays

The measurement of protein biomarkers as indicators of drug toxicity shows promise to improve drug safety and accelerate the development timeline. However, selection of the appropriate combination of protein biomarkers and development of assays to accurately measure them presents a challenge to the community of pharmaceutical toxicologists.

Blood Urea Nitrogen (BUN) and serum creatinine are well established biomarkers of kidney injury. Unfortunately, traditional clinical tests that measure BUN and serum creatinine levels are not suitable for use in drug safety assessment. They are not sensitive enough to detect subtle drug-induced kidney injury and often do not correlate to histochemical measurements of the extent of the damage.

MESO SCALE DISCOVERY® (MSD®) has developed a series of immuno-assays that measure proteins characterized as early-stage, kidney injury biomarkers in humans. Experiments to characterize the assays verify that they are sensitive, specific, and accurate. In addition to meeting these specific requirements for toxicology assays, they also provide the standard advantages of the MSD platform: wide dynamic range (allows endogenous and elevated levels to be measured in a single dilution factor), improved assay throughput (over ELISA and bead-based assay formats), and consistent performance within and across manufacturing lots. The following sections summarize MSD's new human biomarker assays for rapid stratification and accurate localization of kidney injury.

The Significance of Kidney Toxicity Biomarkers



Description of Markers

αGlutathione-S-Transferase (αGST): Urinary αGST is a sensitive and specific biomarker believed to be associated with proximal tubular injury.

Albumin: Damage to the kidney can lead to albuminuria, secretion of albumin into the urine.

β2 microglobulin (β2M): β2M levels may be elevated in kidney failure, inflammation, and certain cancers.

Calcium binding proteins (Calbindin): Calbindin is a distal tubule-specific protein that has been detected in urine under certain pathological conditions.

Clusterin: Increased urinary levels are observed in rat models of tubular proteinuria but not glomerular proteinuria.

Cystatin C: Cycstatin C level is a good indicator of Glomeral Filtrate Rate (GFR) function which, in turn, indicates kidney function.

Epidermal growth factor (EGF): In a variety of experimentally induced forms of acute renal failure, the mRNA and protein levels for kidney EGF fall markedly and remain low for a prolonged period.

Interleukin 18 (IL-18): IL-18 is a proinflammatory cytokine released in response to injury of renal tubular epithelial cells. It may act as an earlier biomarker than serum creatinine in predicting Acute Kidney Injury (AKI) in critically ill adult patients.

Interferon gamma-induced protein 10 kDa (IP-10/CXCL10): IP-10 level in urine is significantly elevated during kidney allograft rejection and diabetic nephropathy.

Neutrophil gelatinase-associated lipocalin (NGAL)/Lipocalin-2: Urine NGAL levels correlate with severity and duration of AKI, length of stay, dialysis requirement, and death. It also provides a way to identify patients at risk of developing potentially severe AKI.

Macrophage migration inhibitory factor (MIF): Increasing levels of MIF in urine correlate with kidney leukocyte accumulation and the severity of renal damage in proliferative forms of glomerulonephritis.

Monokine Induced by Interferon gamma (MIG/CLCL9): MIG is significantly elevated in renal graft recipients with acute rejection, acute tubular injury and BK virus nephritis.

Osteopontin (OPN): Increased urinary levels of OPN has been observed in rat models and humans following nephrotoxicity.

Osteoactivin: Early-phase upregulation of osteoactivin occurs in the tubular epithelium in response to renal injury.

πGlutathione-S-Transferase (πGST): Urinary πGST is a sensitive and specific biomarker for renal distal tubular injury.

Retinol binding protein 4 (RBP4): RBP4 is the specific carrier for retinol and an important marker for kidney abnormality and dysfunction.

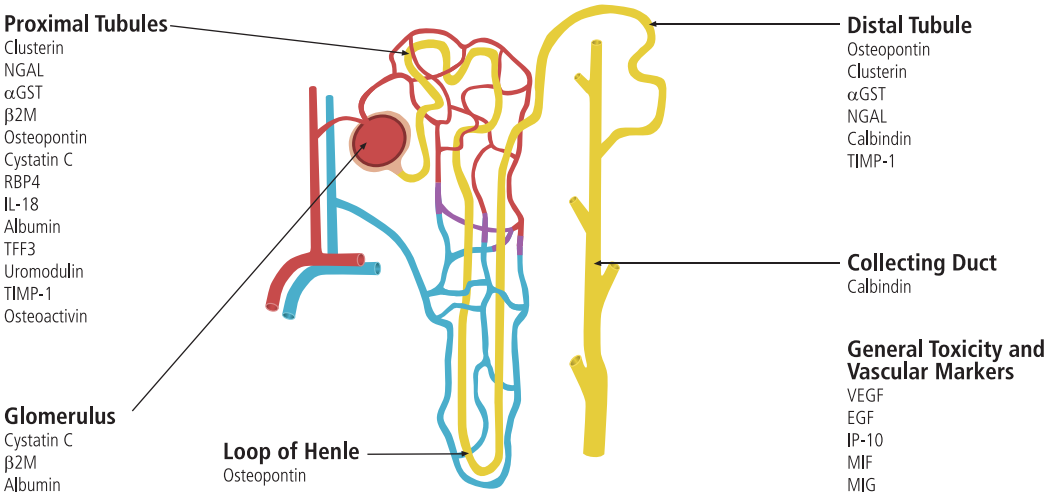
Intestinal trefoil factor (TFF3): A decreased TFF3 level of expression correlates with nephrotoxicity.

Metallopeptidase inhibitor 1 (TIMP-1): Urinary TIMP-1 is increasingly used to indicate biochemical perturbations due to renal toxicity.

Tamm-Horsfall glycoprotein (Uromodulin): Common variants in the region of the UMOD gene, which encodes uromodulin (Tamm-Horsfall protein), have been associated with chronic kidney disease.

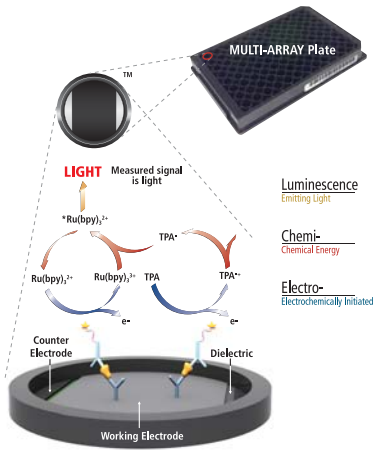
Vascular endothelial growth factor (VEGF): VEGF is upregulated in response to kidney injury.

Human Kidney Injury Biomarkers



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.

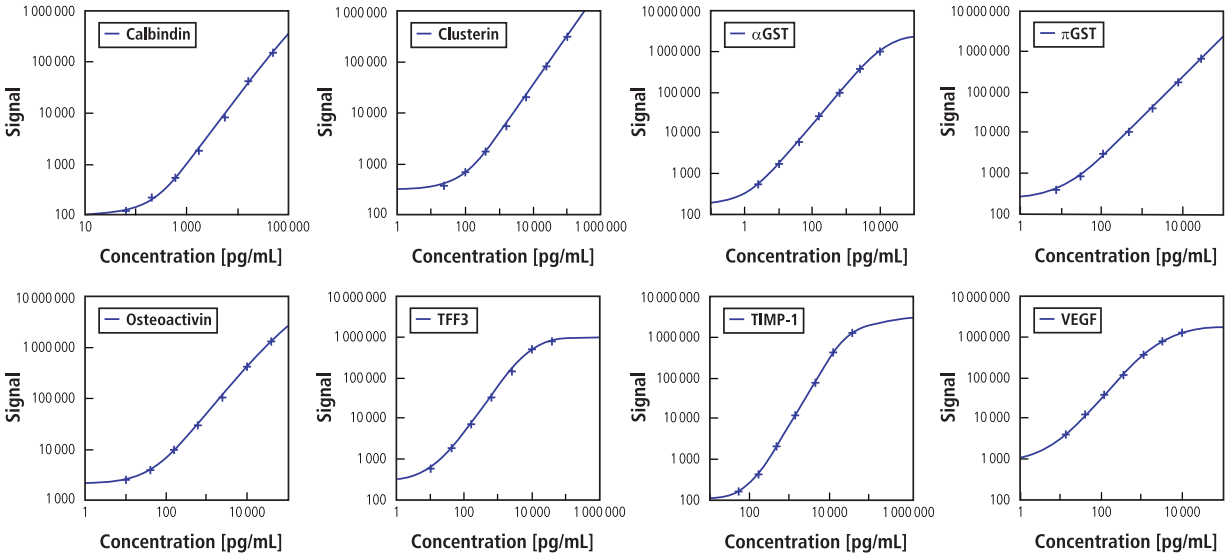


Electrochemiluminescence Features:

- 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, are 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

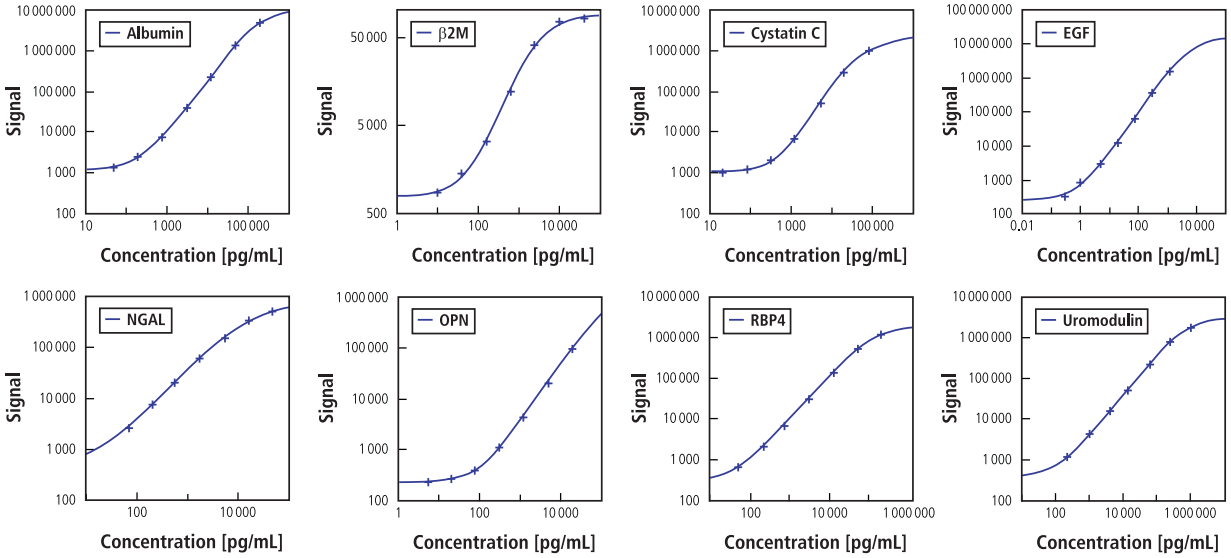
Observed Standard Curve of Human Kidney Biomarkers: Group 1

5- to 20-fold sample dilution is required for the following markers:



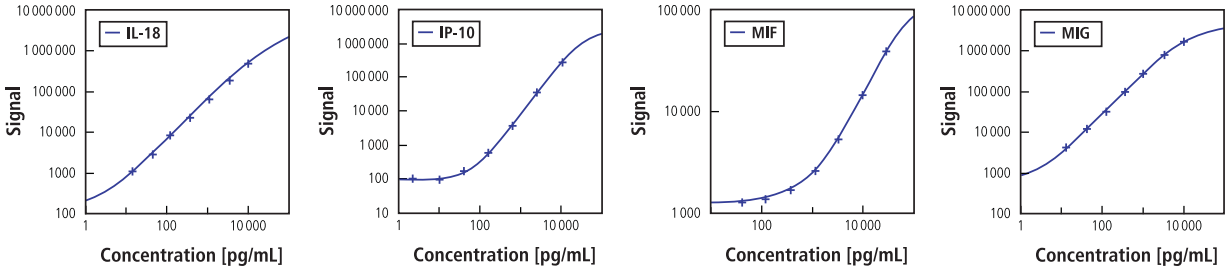
Observed Standard Curve of Human Kidney Biomarkers: Group 2

100- to 1000-fold sample dilution is required for the following markers:



Observed Standard Curve of Human Kidney Biomarkers: Group 3

Samples may be used neat or with minimal dilution for the following markers:



Assay Sensitivity: Comparison to ELISA

MSD assays are ultra-sensitive in complex matrices. Unlike typical ELISA, they can be used for measurement of normal and diseased populations with a single dilution of the sample.

	Group 1		
	LLOD (pg/mL)		Recommended Dilution Fold
	MSD	ELISA	
αGST	0.33	36-90	5 -20
Calbindin	43	-	2 -10
Clusterin	11	189	5 - 20
Osteoactivin	9.0	46	5 - 20
πGST	2.0	156	5 - 20
TFF3	1.2	720	5 - 20
TIMP1	31	80	5 - 20
VEGF	0.24	9.0	5 - 20

	Group 2		
	LLOD (pg/mL)		Recommended Dilution Fold
	MSD	ELISA	
Albumin	33	100000	100 - 1000
EGF	0.090	0.70	100 - 1000
Uromodulin	7.9	5500	100 - 1000
Cystatin C	56	780	100 - 200
NGAL	1.3	12	100 - 200
OPN	214	11-50	100 - 200
RBP4	0.95	224	10 -100
β2M	9.0	100000	20 -100

	Group 3		
	LLOD (pg/mL)		Recommended Dilution Fold
	MSD	ELISA	
IL-18	0.32	12	Neat - 5
IP-10	45	1.7	Neat
MIF	151	7-46	Neat
MIG	0.090	3.8	Neat

Samples

The levels of kidney injury biomarkers were tested in three (3) human pooled urine samples and 12 individual urine samples.

	Concentration (pg/mL)											
Individual Urine Samples	αGST	β2M	Cystatin C	πGST	Calbindin	Clusterin	Osteoactivin	Uromodulin	Albumin	OPN	RBP4	TFF3
1	3.0	56317	62511	4256	1236	15462	437	2187619	15343004	1027615	389	35
2	1817	40855	178743	7802	3444	79214	1209	5780725	2259152	124003	1733	2186
3	109	46319	107687	13140	5661	55238	834	4237668	3445248	267140	554	139
4	76	24301	56682	1570	2425	40734	670	1458033	1330127	1065921	513	9
5	27	25436	122668	5409	10271	93586	1730	5247252	4148917	- [1]	331	23
6	3832	13757	130154	2946	5414	89927	610	3176137	2067008	177438	1377	454
7	1744	28984	180463	15560	7743	59560	1303	6463485	4849257	- [1]	469	134
8	337	1862	10430	5643	1511	13927	352	797386	590070	8141	75	92
9	2750	6998	78910	9956	1696	17974	1086	3163067	1103292	782901	490	595
10	11	2726	230323	21064	6775	240449	1430	- [1]	13869298	- [1]	877	49
11	810	2561	68171	12208	5365	28062	1058	3914141	2756094	- [1]	342	427
12	824	13722	62246	11451	6051	25782	1591	3773711	2730744	- [1]	371	296

[1] Out of quantitative range

	Concentration (pg/mL)							
Pooled Urine Samples	EGF	IL-18	IP-10	MIF-1	MIG-1	NGAL	TIMP-1	VEGF
1	45500	20	85	480	25	159800	32900	187
2	41800	240	-	990	5.0	53900	2300	62
3	14600	12	-	230	3.0	36400	1700	36

Dilution Linearity

To assess linearity, pooled normal urine samples were sequentially diluted. The concentrations shown below have been corrected for dilution (concentration = measured concentration x dilution factor). Percent recovery is calculated as the measured concentration divided by the concentration of the previous dilution (expected). The concentrations shown in blue are outside the quantitative range.

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

2- to 20-fold is the optimal sample dilution for the markers shown in the table below.

	Calbindin				Clusterin				VGEF				TFF3			
Dilution Factor	Conc. (pg/mL)	Average Counts	% Recovery	% CV	Conc. (pg/mL)	Average Counts	% Recovery	% CV	Conc. (pg/mL)	Average Counts	% Recovery	% CV	Conc. (pg/mL)	Average Counts	% Recovery	% CV
1	5881	9894		4.2	3581	15561		8.1	833	118305		13.8	12	636		13.1
2	7698	5674	131	1.6	6089	13216	170	2.1	823	58292	99	9.7	172	4356	1384	4.3
4	8579	2659	111	7.6	6192	6706	102	7.2	847	29684	103	14.3	249	3096	145	3.2
8	8922	1166	104	4.4	5805	3182	94	4.0	857	14844	101	3.4	265	1608	106	2.0
16	10283	606	115	7.2	5829	1664	100	6.9	927	7958	108	5.7	216	685	82	2.7
32	11359	323	110	5.8	6430	991	110	6.6	945	4065	102	11.7	167	335	77	11.2

	Osteoactivin				αGST				πGST				TIMP-1			
Dilution Factor	Conc. (pg/mL)	Average Counts	% Recovery	% CV	Conc. (pg/mL)	Average Counts	% Recovery	% CV	Conc. (pg/mL)	Average Counts	% Recovery	% CV	Conc. (pg/mL)	Average Counts	% Recovery	% CV
1	637	31333		2.1	33	5688		16.5	4322	96211		7.5	534	2745		10.8
2	695	18131	109	2.3	238	23026	729	3.2	10239	114736	237	1.9	1236	3459	231	4.1
4	614	9219	88	9.4	249	11357	104	0.8	11119	60738	109	3.4	1766	2030	143	10.6
8	622	5730	101	2.7	224	4827	90	0.6	9816	25916	88	4.6	1920	801	109	7.9
16	609	3891	98	16.5	210	2191	94	1.9	8701	11155	89	2.6	2252	385	117	7.5
32	590	2980	97	21.4	212	1130	101	2.2	7413	4673	85	1.8	2398	195	107	1.5

100- to 200-fold is the optimal sample dilution for the markers shown in the table below.

	Albumin				NGAL				OPN				RBP4			
Dilution Factor	Conc. (pg/mL)	Average Counts	% Recovery	% CV	Conc. (pg/mL)	Average Counts	% Recovery	% CV	Conc. (pg/mL)	Average Counts	% Recovery	% CV	Conc. (pg/mL)	Average Counts	% Recovery	% CV
8	1036496	435350	140	6.4	52539	178894	98	7.8	662836	292703	103	16.6	7430	28422	104	0.6
16	1772494	386149	171	8.5	57008	109176	109	5.2	658307	185811	99	18.3	6949	13397	94	2.1
32	2277108	268151	128	11.1	55530	57588	97	9.7	903582	136677	137	4.4	6610	6432	95	2.1
64	2590758	161387	114	3.5	54617	29613	98	7.8	930619	72036	103	3.2	6545	3238	99	3.1
128	2672161	85258	103	4.2	52577	14685	96	6.1	903365	32988	97	6.6	6740	1721	103	2.1

	Uromodulin				EGF				β2M				Cystatain C			
Dilution Factor	Conc. (pg/mL)	Average Counts	% Recovery	% CV	Conc. (pg/mL)	Average Counts	% Recovery	% CV	Conc. (pg/mL)	Average Counts	% Recovery	% CV	Conc. (pg/mL)	Average Counts	% Recovery	% CV
8	4220589	1373991	131	2.1	12223	1956109	178	3.1	156725	120949	117	27.4	308942	922910	99	4.9
16	4569273	886183	108	2.5	15192	1150708	124	6.9	164478	109649	105	9.1	341276	524691	110	0.5
32	4555123	500461	100	1.3	14971	521714	99	4.8	198153	94850	120	5.4	363871	246883	107	2.6
64	4262533	253279	94	6.0	14142	225570	94	9.4	195221	67193	99	2.6	378134	102700	104	1.1
128	4382821	135671	103	1.7	14968	110700	106	4.9	184043	37894	94	10.4	391350	41229	103	3.7

Samples should be tested neat for the markers shown in the table below.

	MIG				IP-10				MIF				IL-18			
Dilution Factor	Conc. (pg/mL)	Average Counts	% Recovery	% CV	Conc. (pg/mL)	Average Counts	% Recovery	% CV	Conc. (pg/mL)	Average Counts	% Recovery	% CV	Conc. (pg/mL)	Average Counts	% Recovery	% CV
1	4.2	1306		7.5	85	291		4.4	292	1587		5.6	281	18263		6.2
2	5.1	816	119	2.0	77	155	90	50.7	665	1633	228	1.3	252	8480	90	2.5
4	5.2	464	103	2.1	-	-	-	-	475	1389	72	1.2	223	3927	88	2.7

Conclusions

MSD has developed and characterized a series of human kidney injury biomarker assays that may be used in drug safety assessment. These assays demonstrate sensitive and accurate measurement in complex sample matrices and offer additional benefits over traditional ELISA methods, including wide dynamic range and the opportunity for multiplexing. Some of these assays are available now for purchase from the MSD catalog, while the others may be purchased on a custom basis.