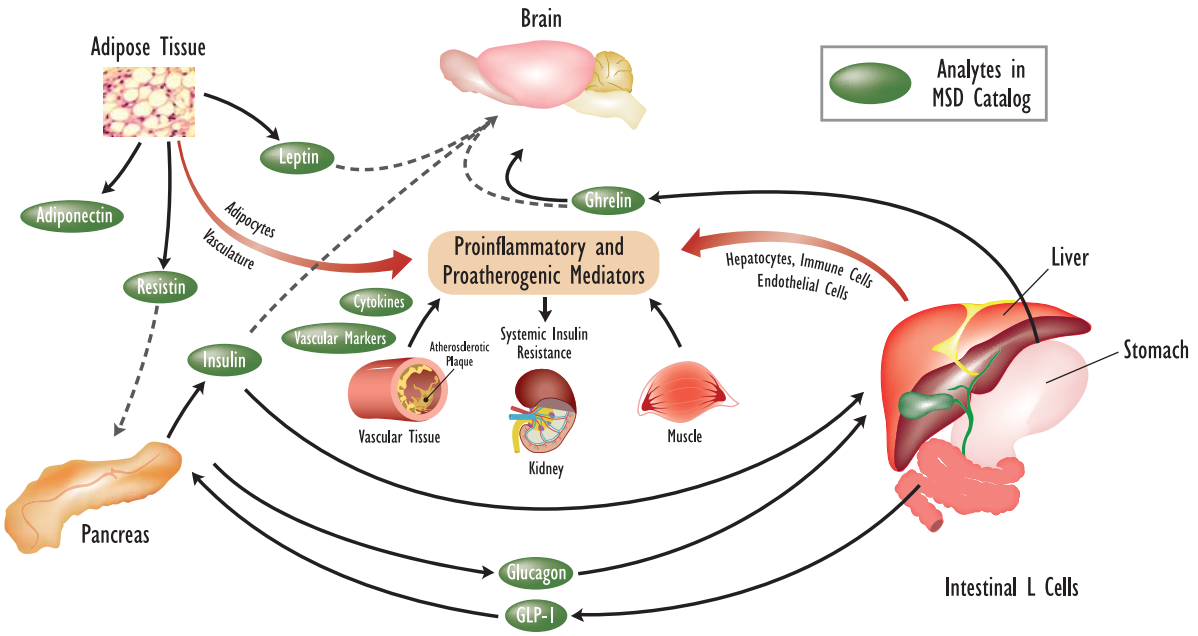
Rodent Biomarker Assays for Obesity, Diabetes, and Metabolic Syndrome

The complex pathology of diabetes, cardiovascular disease and metabolic syndrome has driven the demand for biomarkers related to functional outcomes. Obesity, which has reached epidemic proportions worldwide, is directly related to increased risk of diabetes, hypertension, atherosclerosis and metabolic syndrome. Key organs play a central role in these complex diseases, making invasive, physiological explorations difficult and often impossible in humans. Animal models of diabetes and obesity are therefore critical to finding molecules that regulate blood glucose levels and satiety. Understanding and developing therapeutic strategies that target both adipose inflammation and metabolic dysregulation will be critical measures in rodent studies that are designed to translate quickly to the human clinical condition. MSD has developed quantitative immunoassays that interrogate metabolkine regulators of energy metabolism (Leptin) and glycemic control (Insulin, GLP-1, Glucagon) in serum and plasma samples. These assays, available individually and in multiplex panels, complement an existing selection of MSD metabolic, cytokine and vascular biomarker assays. This broad selection of assays provides for comprehensive and high-throughput, quantitative assessments of biomarkers critical to drug discovery and monitoring clinical interventions in serum and plasma samples.

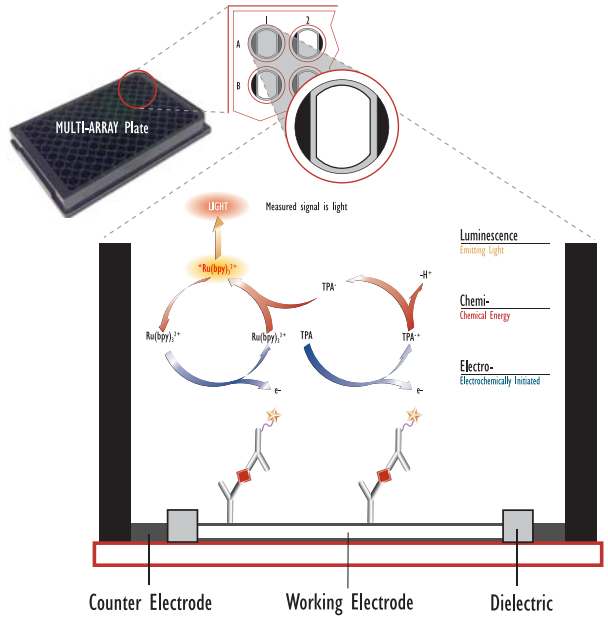
Metabolic Regulators of Glycemic and Appetite Control



MSD MULTI-ARRAY assays are now available for high-throughput, quantitative measurements of metabolic serum and plasma biomarkers

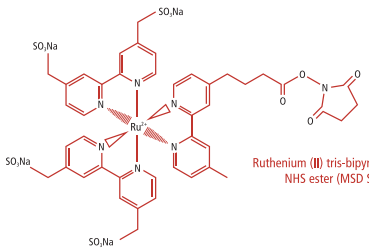
The MSD® Platform

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 background signals and high signal to background ratios - the stimulation mechanism (electricity) is decoupled from the signal (light)
- Proximity - only labels bound near the electrode surface are detected, enabling non-washed assays
- Flexibility - labels are stable, non-radioactive, and are conveniently conjugated to biological molecules
- Emission at ~620 nm - eliminating problems with color quenching
- Signal amplification - multiple excitation cycles of each label enhance light levels and improve sensitivity



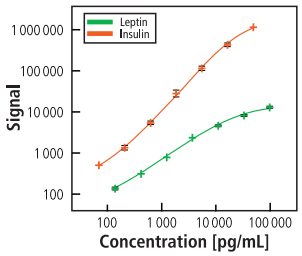
Ruthenium (II) tris-bipyridine-(4-methylsulfonate) NHS ester (MSD SULFO-TAG™ label)

Mouse Metabolic Panel : (Leptin/Insulin)

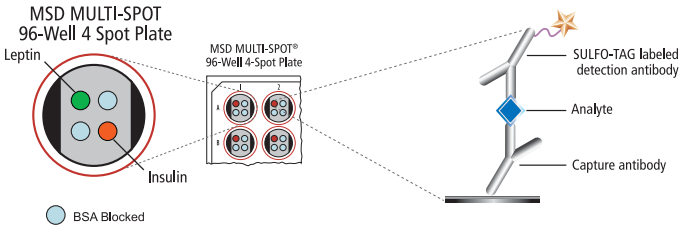
Leptin is a 16 kD product of the ob gene that is produced and released by adipocytes. Leptin plays a key role in metabolism and regulation of adipose tissue and may therefore be a critical regulator of obesity often accompanied by insulin resistance and hyperinsulinemia.

Insulin is a 5.8 kD peptide hormone produced in the pancreas by β-cells of the islets of Langerhans. Its most prominent function is increasing glycogen synthesis by controlling glucose uptake in liver, muscle and adipose tissue.

Standard Curve



The MSD Mouse Metabolic Assay is designed for use with mouse serum and plasma samples. The standard curves demonstrate the dynamic range of the assay.



Endogenous Levels

	Normal	
Sample ID	Leptin (pg/mL)	Insulin (pg/mL)
1	893	4410
2	947	3219
3	1706	13569
4	969	6376
5	2285	3805
6	5962	8302
7	1787	6103
8	612	1167

Endogenous levels of leptin and insulin from individual normal mouse serum samples

Insulin		
Concentration (pg/mL)	Mean	% CV
0	277	3
69	497	1
206	1349	12
617	5663	9
1852	28144	18
5556	114310	12
16667	422598	10
50000	1121247	6

Leptin		
Concentration (pg/mL)	Mean	% CV
0	64	14
137	135	8
412	316	5
1235	785	6
3704	2334	2
11111	4580	5
33333	8235	6
100000	13011	6

Protocol:

1 Add 150 µL Blocking Solution, incubate 1 hour at RT.

2 Wash with PBS-T. Add 40 µL Detection Antibody. Add 10 µL standard/sample, incubate 2 hours at RT.

3 Wash with PBS-T. Add 150 µL of Read Buffer, read.

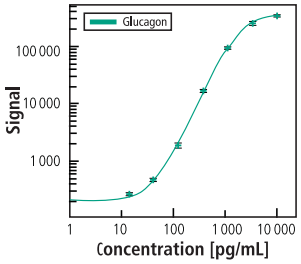
	Leptin	Insulin
LLOD (pg/mL)	43	15

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

Mouse/Rat Glucagon

Glucagon is a 29-residue polypeptide hormone that is produced in the pancreas by the α-cells of the islets of Langerhans. Glucagon is involved in maintaining normal levels of glucose in the blood by acting on liver glycogen, converting it to glucose.

Standard Curve



The MSD Mouse/Rat Glucagon Assay is designed for use with mouse and rat serum and plasma samples. The standard curve demonstrates the dynamic range of the assay.

Glucagon		
Concentration (pg/mL)	Mean	% CV
0	185	13
14	265	3
41	477	3
123	1,864	10
370	16,483	2
1111	94,445	2
3333	249107	8
10000	339538	5

	Glucagon
LLOD (pg/ml)	19

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

Protocol:

1 Add 150 µL Blocking Solution, incubate 1 hour at RT.

2 Wash with PBS-T. Add 20 µL Assay Diluent. Add 40 µL standard/sample, incubate 2 hours at RT.

3 Wash with PBS-T. Add 25 µL of Detection Antibody, incubate 1 hour at RT.

4 Wash with PBS-T. Add 150 µL of Read Buffer, read.

Spike Recovery

	% Recovery
Spiked Serum	68
Spiked Heparin Plasma	86
Spiked EDTAPlasma	88

Serum, heparin plasma, and EDTA plasma were spiked with the calibrators at multiple values throughout the range of the assay. The % recovery is calculated as indicated in the table and the values represent average spike recovery over multiple pooled samples.

% Recovery = measured / expected * 100

GLP-1

Glucagon-like peptide-1 (GLP-1) is a 3.5 kD protein hormone produced in intestinal epithelial endocrine L cells and is associated with lowering blood glucose levels. By activation of different physiological systems, it plays roles in gastric emptying upon intake of nutrients, the regulation of short-term feeding behavior, the stimulation of β-cell proliferation, the promotion of glucose-dependent insulin secretion and insulin biosynthesis, and also the inhibition of glucagon secretion.
MSD offers a comprehensive array of GLP-1 assays that measure both the active and total GLP-1 proteins in mouse/rat serum and plasma samples.

Protocol:

1 Add 150 µL Blocking Solution, incubate 1 hour at RT.

2 Wash with PBS-T. Add 25 µL Assay Diluent. Add 25 µL standard/sample, incubate 2 hours at RT.

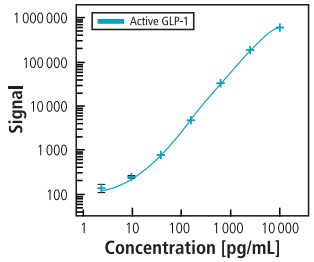
3 Wash with PBS-T. Add 25 µL of Detection Antibody, incubate 1 hour at RT.

4 Wash with PBS-T. Add 150 µL of Read Buffer, read.

Mouse/Rat Active GLP-1

Synthetic GLP-1 (7-36) amide was diluted in a serum-based diluent with specific protease inhibitors to limit degradation. Calibrators, serum and plasma samples were assayed on MSD MULTI-SPOT 96-Well 4 Spot plates coated with anti-GLP-1 antibody specific for amino acid 7 of the GLP-1 protein. GLP-1 (7-36) amide and GLP-1 (7-37) were detected with a blend of MSD SULFO-TAG labeled anti-GLP-1 antibodies to these specific forms. Mean signals are the average of triplicate wells from a representative experiment to generate a standard curve.

Standard Curve



Active GLP-1		
Concentration (pg/mL)	Mean	% CV
0	93	22
2.4	136	21
9.8	245	6
39	763	3
156	4,774	3
625	32430	4
2500	185206	1
10000	592615	3

	Active GLP-1
LLOD (pg/ml)	6

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

Dilutional Linearity

Measured spiked analyte levels in pooled mouse plasma followed by subsequent dilution.

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

	Serum	EDTA Plasma	Heparin Plasma
Fold Dilution	% Recovery	% Recovery	% Recovery
2	111	110	102
4	110	103	101
8	97	75	88

Spike Recovery

Serum, heparin plasma, and EDTA plasma were spiked with the calibrators at multiple values throughout the range of the assay. The % recovery is calculated as indicated in the table and the values represent average spike recovery over multiple pooled samples.

% Recovery = measured / expected * 100

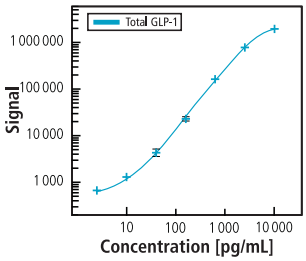
	Spike Conc. (pg/mL)	% Recovery
Spiked Serum	10	85
	100	87
	800	119
Spiked Heparin Plasma	10	88
	100	95
	800	107
Spiked EDTA Plasma	10	98
	100	111
	800	110

There are numerous proteases in serum and plasma that may cause degradation of GLP-1. Blood samples should be prepared into tubes containing 0.1mM diprotin A and 500 KIU aprotinin per mL of whole blood.

Mouse/Rat Total GLP-1

Synthetic GLP-1 (7-36) amide was diluted in a serum-based diluent with specific protease inhibitors to limit degradation. Calibrators, serum and plasma samples were assayed on MSD MULTI-SPOT 96-Well 4 Spot plates coated with anti-total-GLP-1 antibody. All forms of GLP-1 were detected with a blend of MSD SULFO-TAG labeled anti-GLP-1 antibodies. Mean signals are the average of triplicate wells from a representative experiment to generate a standard curve.

Standard Curve



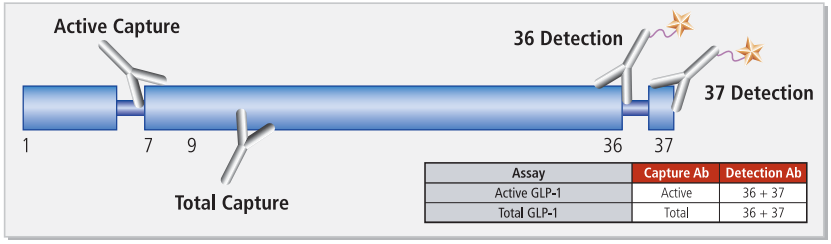
Total GLP-1		
Concentration (pg/mL)	Mean	% CV
0	516	6
2.4	676	1
9.8	1310	2
39	4367	19
156	22905	13
625	159175	5
2500	750260	5
10000	1909444	1

	Total GLP-1
LLOD (pg/ml)	2

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

Antibodies Recognition Site

GLP-1 (7-36)amide and GLP-1 (7-37) are the biologically active forms of GLP-1. In vivo, the amidated form is rapidly degraded by dipeptidyl peptidase IV (DPP IV). Assays for active GLP-1 utilize a capture antibody specific to the 7th amino acid of the GLP-1 protein and detection antibodies specific for the C-terminal, 36th and/or 37th amino acids. Total assays detect all isoforms of GLP-1 present in the sample.



Assay	Capture Ab	Detection Ab
Active GLP-1	Active	36 + 37
Total GLP-1	Total	36 + 37

Schematic of the antibodies recognition sites on GLP-1 protein amino acids 1 - 37.

Cross-Reactivity

The cross-reactivity shown below is calculated based on signal generated using different GLP-1 isoforms.

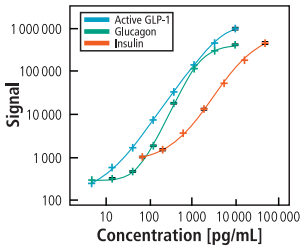
	Cross-Reactivity	
Form	Active GLP-1 Assay	Total GLP-1 Assay
GLP-1 (7-36)amide	100%	100%
GLP-1 (9-36)amide	< 0.1%	100%
GLP-1 (1-36)amide	< 0.1%	100%
GLP-1 (7-37)	100%	100%
GLP-1 (1-37)	< 0.1%	100%

Multiplexing with Metabolic Markers

MSD offers multiplex metabolic assays with similar performance and sensitivity as singleplex assays. Our multiplex assays can be used to limit sample volume and to reduce testing time by generating large data sets for multiple assays.

Mouse/Rat Active GLP-1, Insulin, Glucagon

Standard Curve



Active GLP-1		
Concentration (pg/mL)	Mean	% CV
0	245	5
14	574	3
41	1680	5
123	7524	1
370	33600	2
1111	144054	2
3333	462098	2
10000	1001507	7

Glucagon		
Concentration (pg/mL)	Mean	% CV
0	273	4
14	319	4
41	462	4
123	1860	2
370	18278	3
1111	118169	1
3333	307048	2
10000	428295	2

Insulin		
Concentration (pg/mL)	Mean	% CV
0	877	3
69	1017	3
206	1483	4
617	3665	2
1852	13441	3
5556	53815	2
16667	188004	1
50000	464754	6

	Active GLP-1	Glucagon	Insulin
LLOD (pg/ml)	6	38	19

Spike Recovery

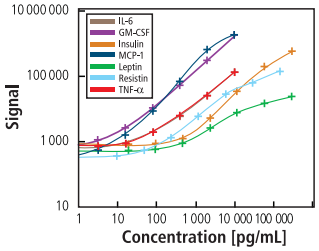
		Average % Recovery		
	Spike Level	Active GLP-1	Glucagon	Insulin
Serum	Low	64	78	111
	Medium	73	94	81
	High	73	98	78
EDTA Plasma	Low	68	83	109
	Medium	87	111	106
	High	109	137	114
Heparin Plasma	Low	77	82	87
	Medium	91	106	93
	High	95	113	93

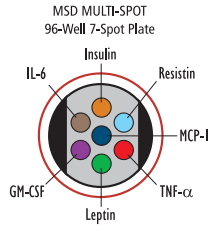
Dilutional Linearity

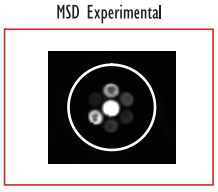
		Average % Recovery		
	Dilution Factor	Active GLP-1	Glucagon	Insulin
Serum	1/2	110	102	116
	1/4	113	100	117
	1/8	109	98	119
EDTA Plasma	1/2	104	97	92
	1/4	107	79	83
	1/8	85	83	74
Heparin Plasma	1/2	103	95	104
	1/4	99	94	101
	1/8	96	113	94

Mouse/Rat Metabolkine Panel

Standard Curve







MSD MULTI-ARRAY technology allows for simultaneous measurement of Metabolic, Cytokine and Vascular biomarkers in multiplex with excellent performance compared to individual assays.

	Detection Limits	
Assay	Multiplex pg/mL	Historical pg/mL
IL-6	4.3	7.7
GM-CSF	1.1	4.4
Insulin	125	~100
MCP-1	1.3	9
Leptin	83	~100
Resistin	10	<1
TNF-α	6.1	3.4

Spike Recovery

Assay	Native Levels		% Recovery					
			High Spike		Mid Spike		Low Spike	
	Serum pg/mL	Plasma pg/mL	Serum	EDTA Plasma	Serum	EDTA Plasma	Serum	EDTA Plasma
IL-6	17	30	125	95	103	93	106	66
GM-CSF	0	0	102	102	102	90	97	80
Insulin	1772	2816	85	122	80	109	95	128
MCP-1	65	44	102	95	100	92	nd	nd
Leptin	3806	1224	118	95	92	70	94	121
Resistin	42760	17800	114	112	121	131	129	136
TNF-a	3	4	102	125	107	134	102	107

Conclusions

- We present highly specific individual and multiplex assays for the detection of plasma and serum biomarkers critical to Diabetes, Obesity and Metabolic Syndrome.
- MSD offers a collection of GLP-1 assays that allow quantification of all forms of this critical metabolic regulator.
- We show the ability to multiplex these assays with other cytokine, vascular and serum biomarkers related to inflammatory states, enabling multiple analytes to be assayed simultaneously in a single well.
- MULTI-ARRAY technology-based assays reduce consumption of precious samples relative to existing technologies.
- Spiked analytes in samples are recovered at the expected levels.
- MSD MULTI-SPOT technology provides highly quantitative and sensitive immunoassays with broad dynamic range that are superior to existing techniques.

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