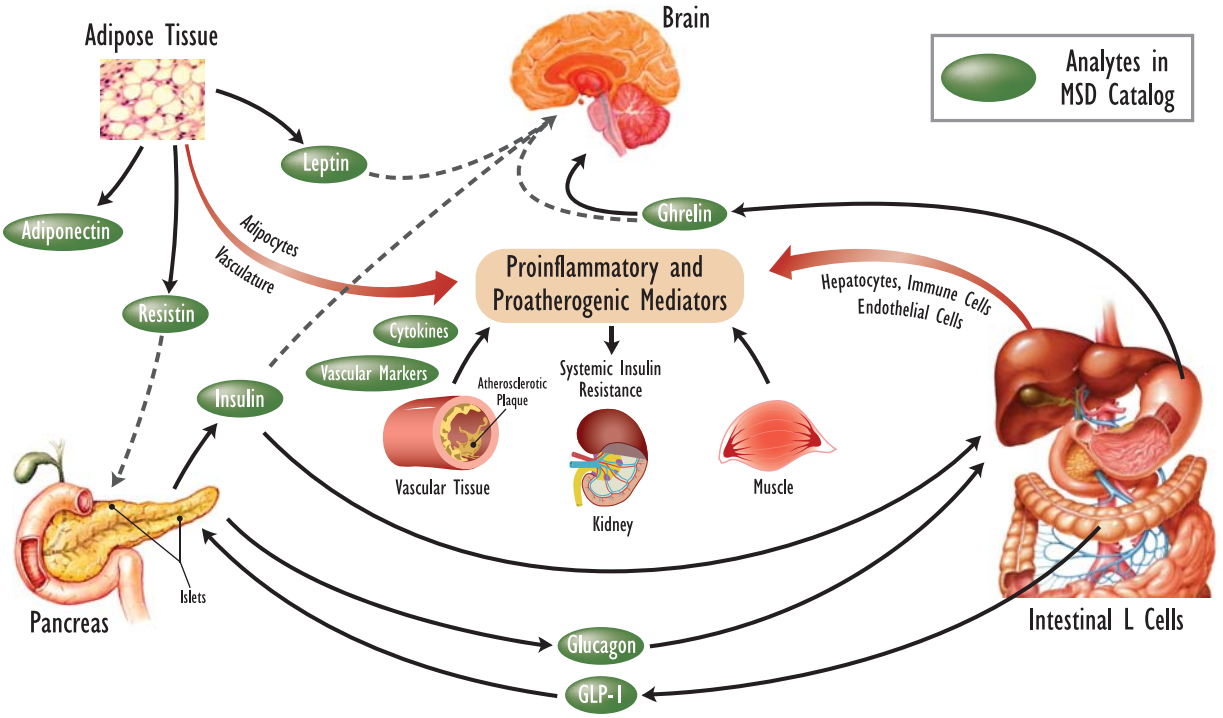
Human Biomarker Assays for Obesity, Diabetes, and Metabolic Syndrome

Obesity, which has reached epidemic proportions worldwide, leads to increased risk for diabetes, hypertension, atherosclerosis and metabolic syndrome. The complexity of these pathologies has driven an increased demand for quantitative measurement of biomarkers linked to these disease states. Proteomics has helped define key serum biomarkers produced in the gut and adipose tissue that change levels in these obesity-related pathologies. Meso Scale Discovery® (MSD) has developed quantitative immunoassays for a number of these markers including metabolic, cytokine and vascular biomarkers assays. Available individually and in multiplex panels, these assays are powerful tools to interrogate the biochemical regulation of energy metabolism and glycemic control in complex biological samples such as human serum and plasma. A number of multiplex products are available including metabolic, cytokine and metabokine panels. This broad selection of assays provides for comprehensive and quantitative assessments of biomarkers critical for drug discovery research and monitoring clinical interventions in Obesity and Diabetes.

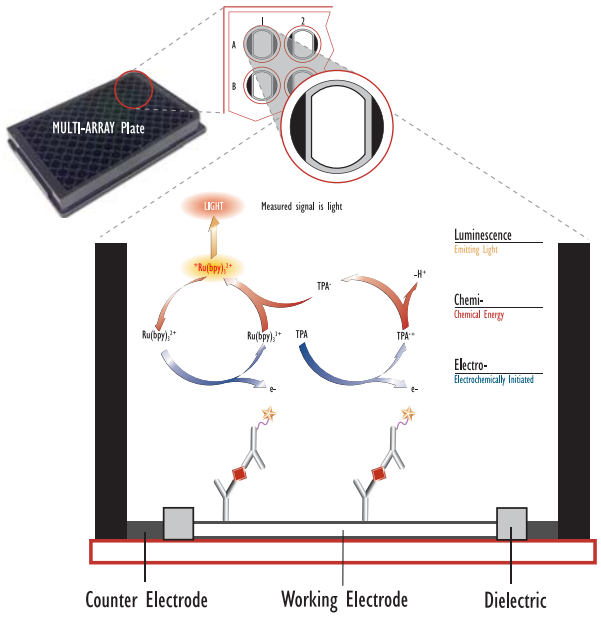
Metabolic Regulators of Glycemic and Appetite Control



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

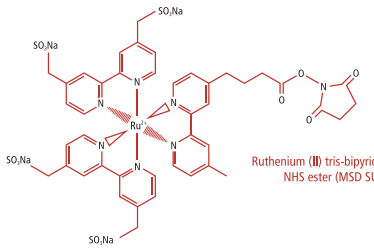
The MSD® Platform

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:

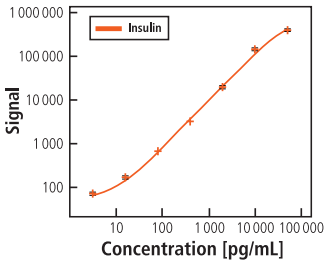
- Minimal background signals and high signal to background ratios - the stimulation mechanism (electricity) is decoupled from the signal (light)
- Proximity - only labels bound near the electrode surface are detected, enabling non-washed assays
- Flexibility - labels are stable, non-radioactive, and 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



Insulin

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

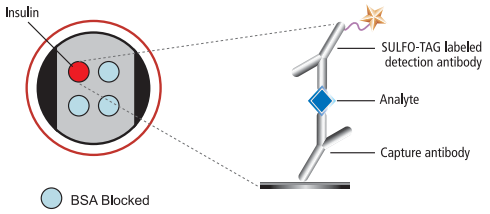


The MSD Human Insulin Assay is designed for use with human serum and plasma samples. The standard curve demonstrates the dynamic range of the assay.

Insulin		
Conc. (pg/mL)	Average Counts	% CV
0	44	9
3	71	5
16	165	11
80	653	3
400	3165	5
2000	18999	7
10000	140326	8
50000	385697	6

	Insulin
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.



Protocol:

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

2 Wash with PBS-T. Add 25 µL Detection Antibody. Add 25 µL standard/sample, incubate 2 hours, RT.

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

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	500	97
	2500	93
	5000	99
Spiked Heparin Plasma	500	88
	2500	85
	5000	83
Spiked EDTA Plasma	500	97
	2500	95
	5000	95

Dilutional Linearity

Measured spiked analyte levels in pooled human serum and plasma followed by subsequent dilution.

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

	Serum	EDTA Plasma	Heparin Plasma
Fold Dilution	% Recovery	% Recovery	% Recovery
2	95	95	98
4	84	86	97
8	87	97	102

Endogenous Levels

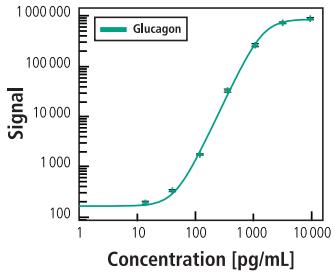
Endogenous levels of human insulin in five matched individual serum and plasma samples were measured in pg/mL.

Sample ID	Serum	EDTA Plasma	Heparin Plasma
1	395	624	470
2	2311	3963	2694
3	58	73	67
4	58	100	78
5	218	393	264

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 Human Glucagon Assay is designed for use with human serum and plasma samples. The standard curve demonstrates the dynamic range of the assay.

Glucagon		
Conc. (pg/mL)	Average Counts	% CV
0	152	12
14	196	8
41	342	5
123	1781	3
370	32710	7
1111	258830	7
3333	717347	4
10000	867267	5

	Glucagon
LLOD (pg/mL)	27

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

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	100	64
	500	70
	1000	70
Spiked Heparin Plasma	100	85
	500	80
	1000	82
Spiked EDTA Plasma	100	88
	500	79
	1000	83

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 human 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.

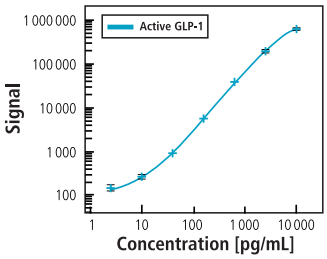
3 Wash with PBS-T. Add 25 µL of Detection Antibody, incubate 1 hour at RT.

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

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		
Conc. (pg/mL)	Average Counts	% CV
0	105	22
2.4	147	16
9.8	264	12
39	912	4
156	5620	4
625	39212	7
2500	195297	7
10000	634910	5

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

	Active GLP-1
LLOD (pg/ml)	5

Dilutional Linearity

Measured spiked analyte levels in pooled human serum and plasma followed by subsequent dilution.

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

	Serum	EDTA Plasma	Heparin Plasma
Fold Dilution	% Recovery	% Recovery	% Recovery
2	105	96	106
4	111	91	107
8	81	65	70

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

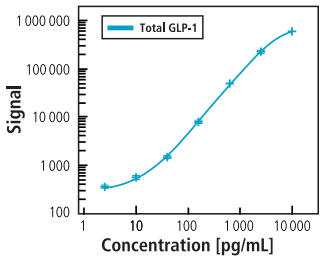
	Spike Conc. (pg/mL)	% Recovery
Spiked Serum	10	100
	100	104
	800	106
Spiked Heparin Plasma	10	99
	100	100
	800	113
Spiked EDTA Plasma	10	93
	100	108
	800	100

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.

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		
Conc. (pg/mL)	Average Counts	% CV
0	281	6
2.4	356	7
9.8	549	7
39	1454	7
156	7794	7
625	48083	5
2500	218425	6
10000	575441	5

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

	Total GLP-1
LLOD (pg/ml)	2

Spike Recovery

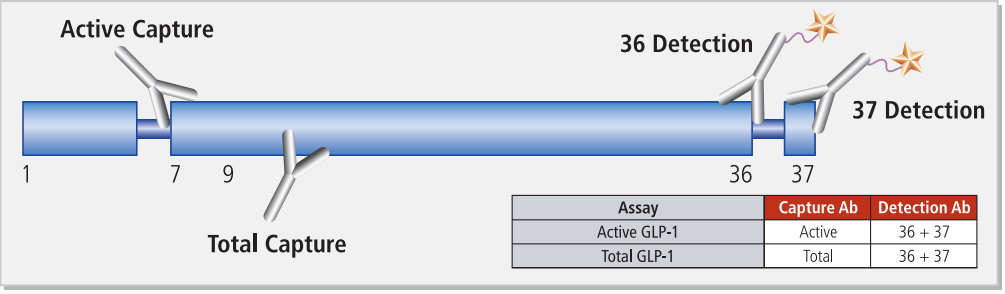
Serum, heparin plasma, and EDTA plasma were spiked with the calibrators at multiple values throughout the range of the assay. The % recovery is calculated as indicated in the table and the values represent average spike recovery over multiple pooled samples.

% Recovery = measured / expected * 100

	Spike Conc. (pg/mL)	% Recovery
Spiked Serum	10	85
	100	98
	800	92
Spiked Heparin Plasma	10	106
	100	92
	800	113
Spiked EDTA Plasma	10	109
	100	101
	800	119

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 GLP-1 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

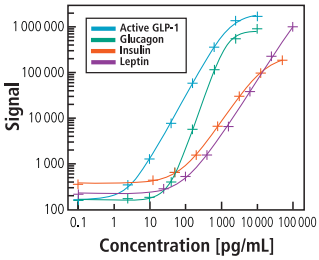
The cross-reactivity shown below is calculated based on signal generated using different GLP-1 isoforms.

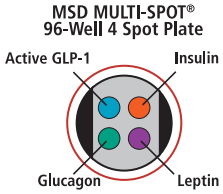
	Cross-Reactivity	
Form	Active GLP-1 Assay	Total GLP-1 Assay
GLP-1 (7-36)amide	100%	100%
GLP-1 (9-36)amide	< 0.1%	100%
GLP-1 (1-36)amide	< 0.1%	100%
GLP-1 (7-37)	100%	100%
GLP-1 (1-37)	< 0.1%	100%

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.

Standard Curve





	Active GLP-1	Insulin	Glucagon	Leptin
LLOD (pg/mL)	1	11	21	61

Active GLP-1		
Concentration (pg/mL)	Average Counts	% CV
0	162	10
2.4	345	1
9.8	1262	2
39.1	7806	1
156	57503	1
625	356515	1
2500	1345265	1
10000	1698466	1

Insulin		
Concentration (pg/mL)	Average Counts	% CV
0	362	3
12.2	437	7
49	644	4
195	1563	1
781	6666	5
3125	29947	4
12500	96447	1
50000	183660	7

Glucagon		
Concentration (pg/mL)	Average Counts	% CV
0	156	5
2.4	168	1
9.8	186	3
39.1	400	1
156	5724	4
625	113210	6
2500	547138	3
10000	899803	2

Leptin		
Concentration (pg/mL)	Average Counts	% CV
0	211	8
24.4	285	2
98	525	1
391	1572	0
1563	6585	5
6250	38369	3
25000	225767	1
100000	988180	4

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.
- MULTI-ARRAY technology-based assays reduce consumption of precious samples relative to existing technologies.
- Spiked analytes in the human 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.