



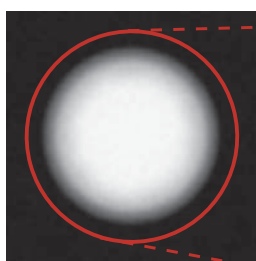
Metabolic Marker Assays: Adiponectin, GLP-1, Insulin, Leptin and Resistin

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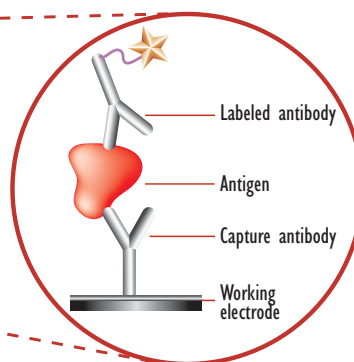
We have developed assays for metabolic markers adiponectin (mouse), GLP-1 (mouse, rat, human), insulin (mouse, rat, human), leptin (mouse, rat, human), and resistin (mouse, rat) on the MSD[®] MULTI-ARRAY[™] platform for measurement in serum or plasma. All assays are sandwich immunoassays using electrochemiluminescent-labeled detection antibodies. The assay protocols, with the exception of rat leptin and mouse/rat resistin, involve a single 1 hour incubation followed by a wash step. Rat leptin and mouse/rat resistin require two incubation steps. Assays are performed in MSD MULTI-ARRAY 96-well single-spot plates and are read on MSD's SECTOR[™] instruments. All assays are sensitive and exhibit a broad dynamic range. These metabolic markers can be multiplexed into panels for simultaneous measurements. A duplex panel of mouse insulin and leptin is demonstrated as an example.

Background and Assay Format

All metabolic analyte assays are formatted as sandwich immunoassays, using either monoclonal – monoclonal, monoclonal – polyclonal, or polyclonal – polyclonal antibody pairs. Detection antibodies are labeled with a $\text{Ru}(\text{bpy})_3^{2+}$ compound, and electrochemiluminescence is used as the detection technology.



ECL image of a 96-well small spot plate.
Light is generated by label on detection antibody on the surface.

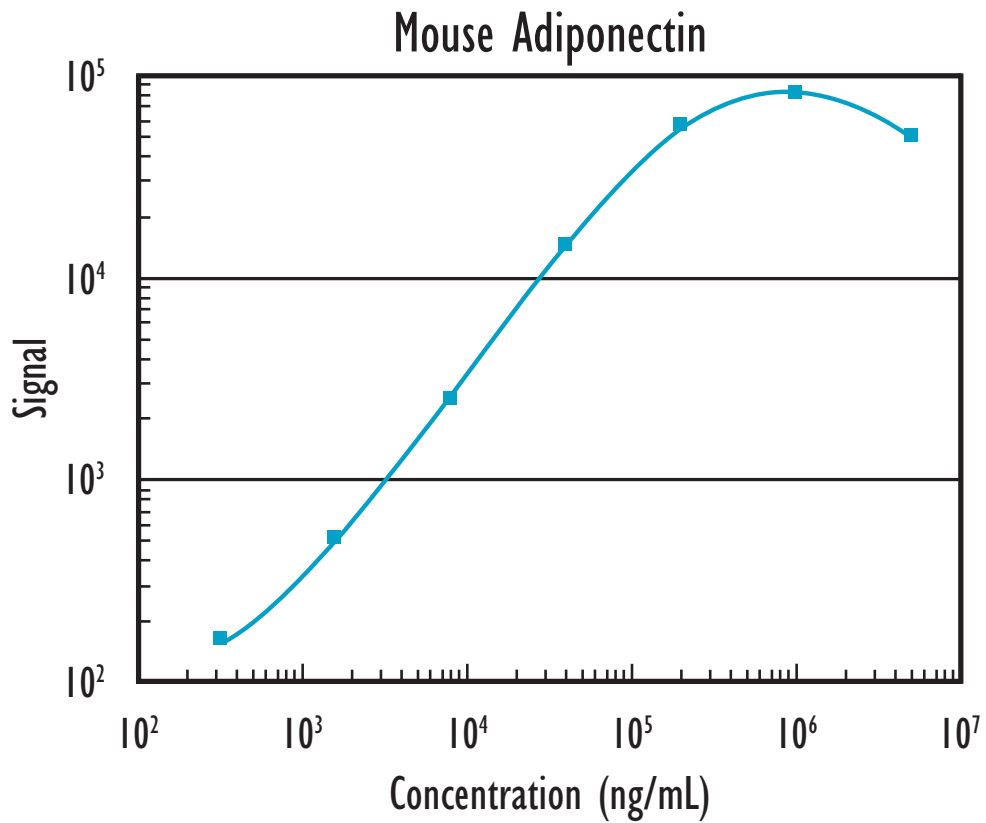


Metabolic Markers - Assay Protocols

Marker	Total Assay Time*	Number of Washes*	Sample Volume Required per Well
Mouse Adiponectin	2 hours	1	10 μ L of pre-diluted (1:1000) sample
GLP-1	2 hours	1	25 μ L
Human Insulin	2 hours	1	25 μ L
Mouse/Rat Insulin	2 hours	1	5 μ L
Human Leptin	2 hours	1	10 μ L
Mouse Leptin	2 hours	1	5 μ L
Rat Leptin	3 hours	2	10 μ L
Mouse/Rat Resistin	3 hours	2	10 μ L of pre-diluted (1:20) sample

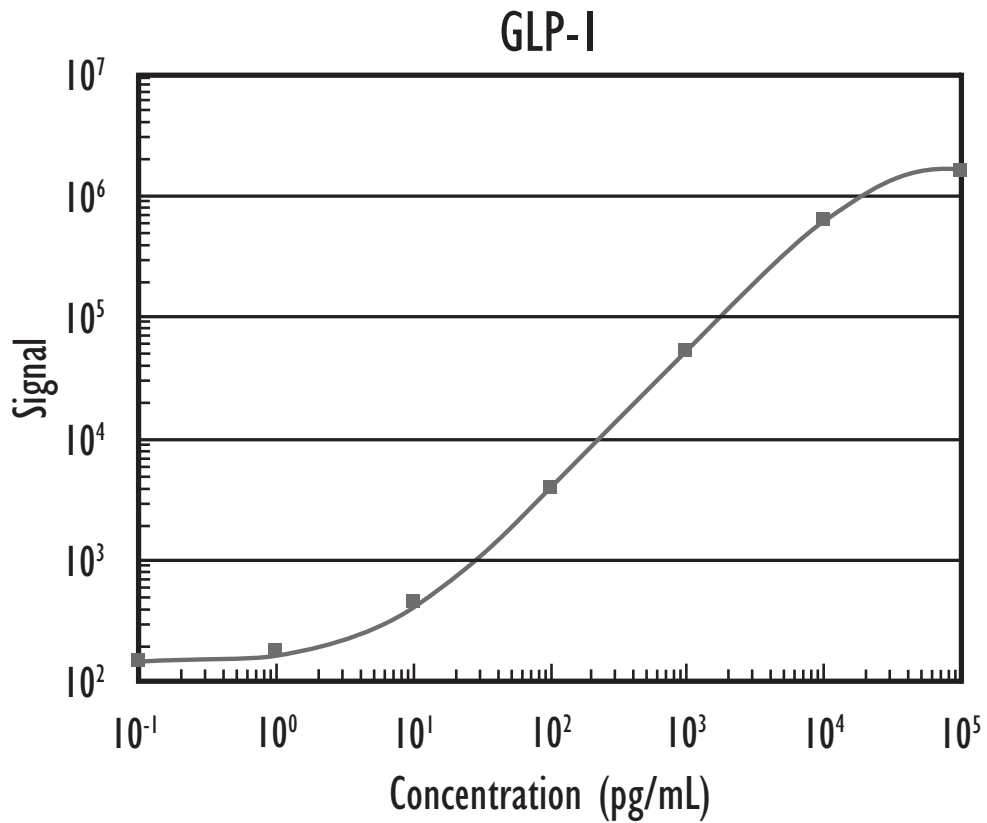
* Not including optional blocking step which is a 1 hr incubation with a blocker solution followed by a wash.

Mouse Adiponectin Assay Performance



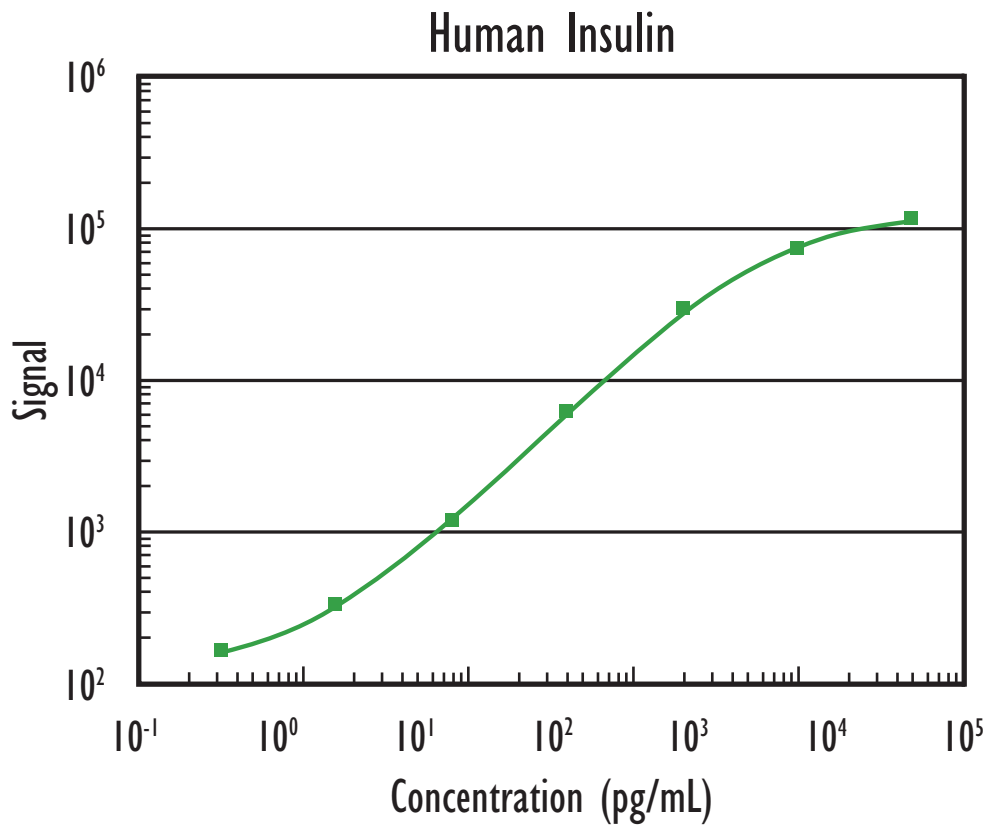
Measurement Range: 100 - 200,000 ng/mL
Variability: less than 8% CV

GLP-1 Assay Performance



Measurement Range: 3 - 10,000 pg/mL
Variability: less than 10% CV

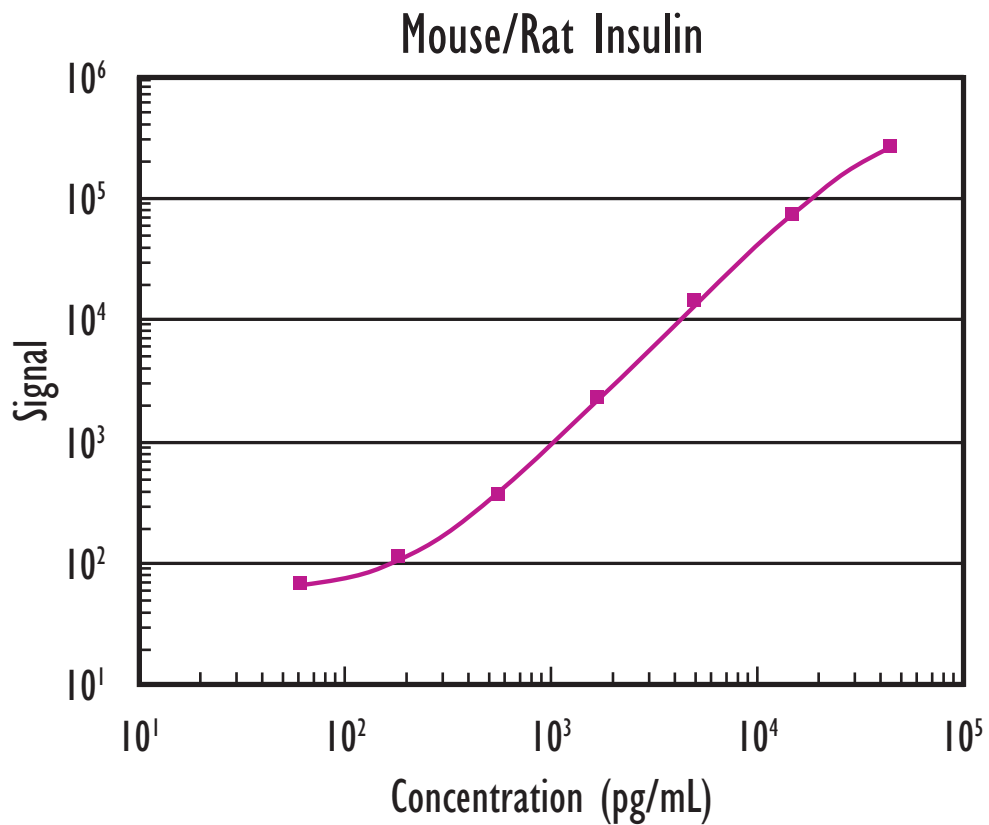
Human Insulin Assay Performance



Measurement Range: 3 - 10,000 pg/mL
Variability: less than 5% CV

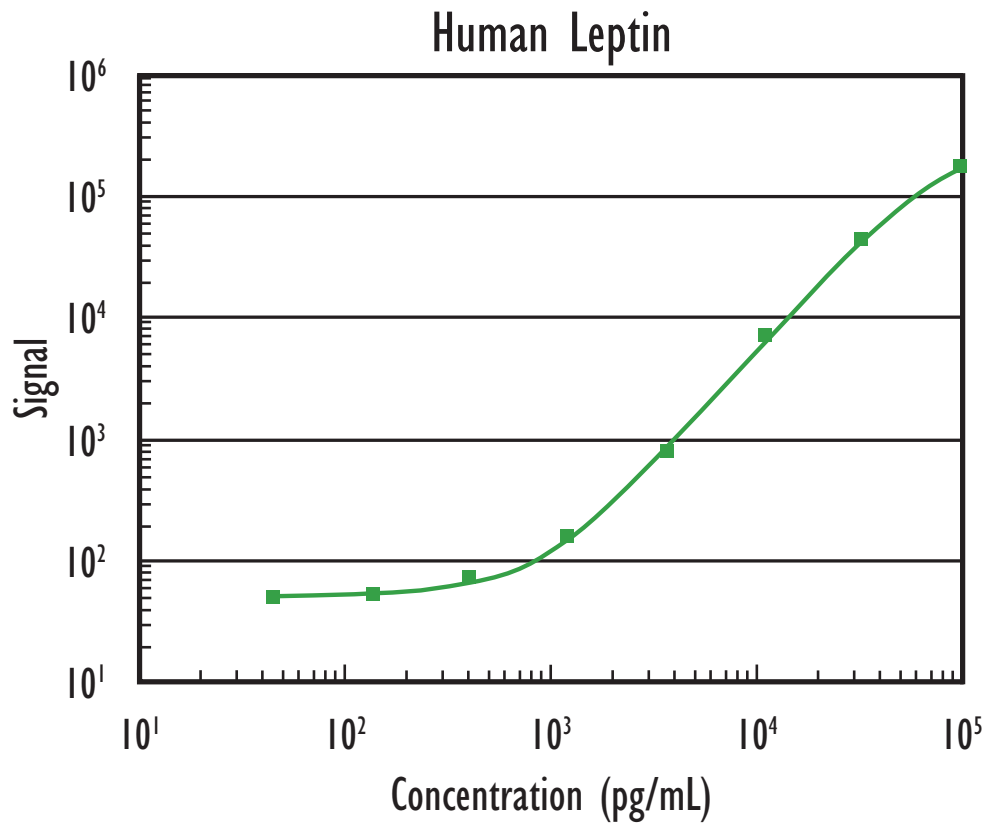
MSD Human Insulin calibrators have been calibrated against the NIBSC (National Institute for Biological Standards and Control) standard.

Mouse / Rat Insulin Assay Performance



Measurement Range: 143 - 50,000 pg/mL
Variability: less than 7% CV

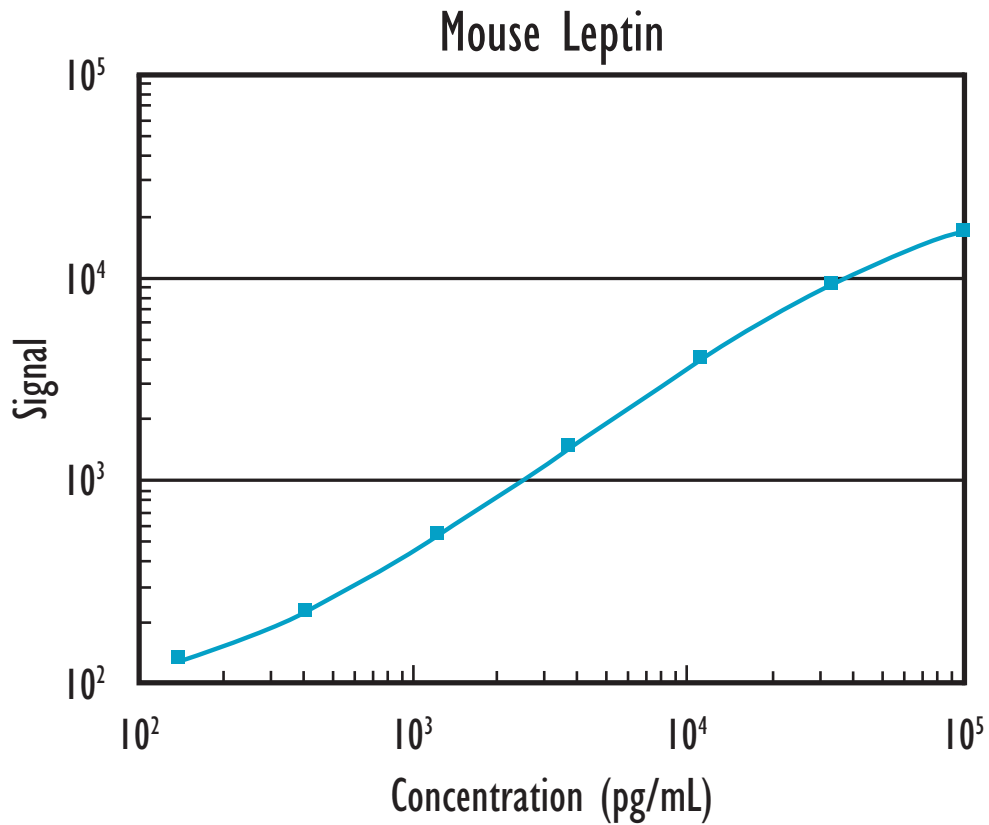
Human Leptin Assay Performance



Measurement Range: 622 - 40,000 pg/mL
Variability: less than 10% CV

MSD Human Leptin calibrators have been calibrated against the NIBSC (National Institute for Biological Standards and Control) standard.

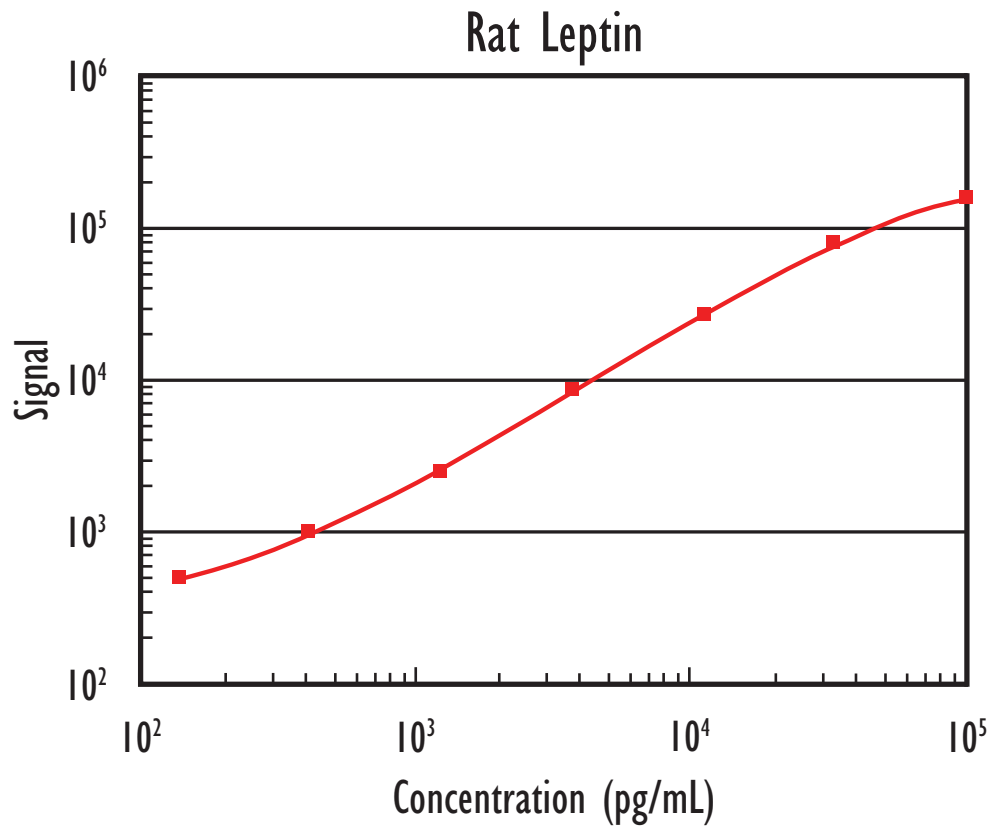
Mouse Leptin Assay Performance



Measurement Range: 146 - 60,000 pg/mL
Variability: less than 10% CV

MSD Mouse Leptin calibrators have been calibrated against the NIBSC (National Institute for Biological Standards and Control) standard.

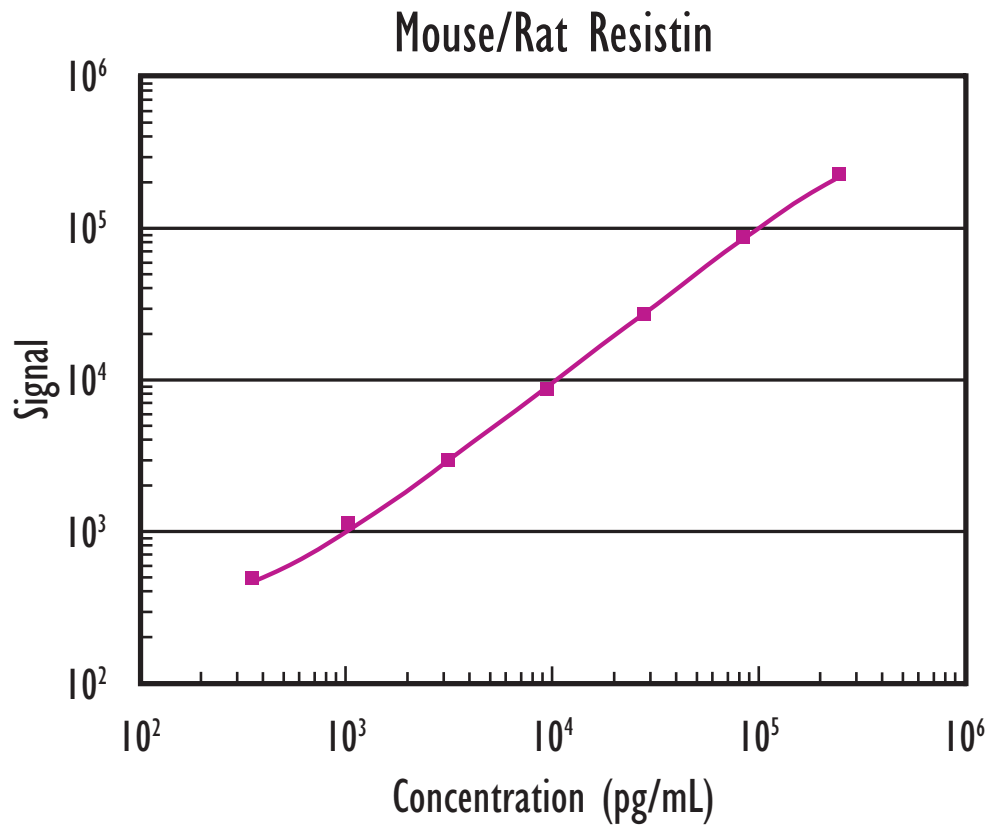
Rat Leptin Assay Performance



Measurement Range: 40 - 30,000 pg/mL

Variability: less than 10% CV

Mouse / Rat Resistin Assay Performance



Measurement Range: 72 - 250,000 pg/mL
Variability: less than 5% CV

Evaluation of MSD Metabolic Marker Assays

Marker	MSD Analytical Sensitivity and Range	ELISA / RIA Analytical Sensitivity	Expected Range	Notes
Mouse Adiponectin	100 ng/mL - 200 µg/mL	500 ng/mL (ELISA)	3,000 - 15,000 ng/mL	MSD assay detects monomeric and trimeric forms of adiponectin.
GLP-1	3 pg/mL - 10 ng/mL	8 pg/mL (RIA)	50 - 170 pg/mL in mice; lower in rats	MSD assay is specific for C-terminus of peptide.
Human Insulin	3 pg/mL - 10 ng/mL	7 pg/mL (RIA)	175 - 525 pg/mL fasting range	
Mouse/Rat Insulin	0.14 ng/mL - 50 ng/mL	0.2 ng/mL (ELISA)	0.5 - 10 ng/mL	There is also a rat RIA insulin kit with a sensitivity of 0.02 ng/mL with a 100 µL sample.
Human Leptin	0.6 ng/mL - 40 ng/mL	0.05 ng/mL (RIA)	3 - 80 ng/mL (large gender dichotomy)	RIA assay range goes to 10 ng/mL with a sample volume of 100 µL.
Mouse Leptin	0.15 ng/mL - 60 ng/mL	0.05 ng/mL (RIA)	1 - 8 ng/mL	Levels can go up to 75 ng/mL for pregnant mice.
Rat Leptin	0.04 ng/mL - 30 ng/mL	0.5 ng/mL (RIA)	1 - 10 ng/mL	
Mouse/Rat Resistin	0.07 ng/mL - 250 ng/mL	0.78 ng/mL (ELISA)	1 - 12 ng/mL	ELISA assay specific only for mouse resistin.

Validation of Metabolic Marker Assays

Spike Recovery

Spike recovery levels were calculated for low, medium and/or high spike levels added to human, mouse or rat serum samples. Average recovery values are tabulated below. EDTA and Lithium Heparin plasma samples yielded similar results demonstrating MSD assay compatibility with common anti-coagulants.

Marker	N	Average Recovery of Low Spike	Average Recovery of Medium Spike	Average Recovery of High Spike
Mouse Adiponectin	10	---	---	107%
GLP-1	8 (h), 4 (m,r)	---	---	80% (h), 92% (m), 80% (r)
Human Insulin	4	94%	98%	109%
Mouse/Rat Insulin	8 (m), 8 (r)	---	---	101% (m), 97% (r)
Human Leptin	4	93%	98%	92%
Mouse Leptin	8	---	---	95%
Rat Leptin	4	89%	100%	111%
Mouse/Rat Resistin	4 (m), 4 (r)	106% (m), 93% (r)	119% (m), 83% (r)	108% (m), 87% (r)

Validation of Metabolic Marker Assays (continued)

Dilution Linearity

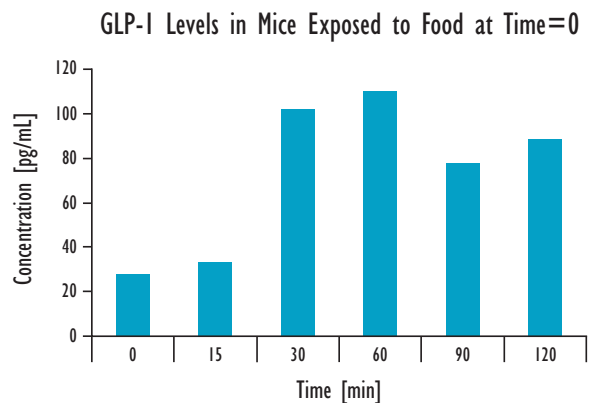
Human, mouse and rat serum/plasma (EDTA and Lithium Heparin) samples were serially diluted and linearity of dilution was assessed for each analyte. Average recoveries for serum samples are tabulated below. Plasma samples exhibited a very similar dilution profile.

Marker	N	Recovery at 1/4 Dilution	Recovery at 1/2 Dilution	Recovery at 3/4 Dilution
Mouse Adiponectin	3	105%	106%	107%
GLP-I	3 (h, m, r)	102% (h), 118% (m), 124% (r)	120% (h), 105% (m), 117% (r)	113% (h), 100% (m), 103% (r)
Human Insulin	3	113%	104%	99%
Mouse/Rat Insulin	3 (m), 2 (r)	94% (m), 95% (r)	99% (m), 112% (r)	96% (m), 104% (r)
Human Leptin	3	113%	105%	105%
Mouse Leptin	3	97%	108%	104%
Rat Leptin	3	118%	109%	100%
Mouse/Rat Resistin	3 (m, r)	109% (m), 88% (r)	105% (m), 91% (r)	102% (m), 98% (r)

Applications of MSD Metabolic Marker Assays

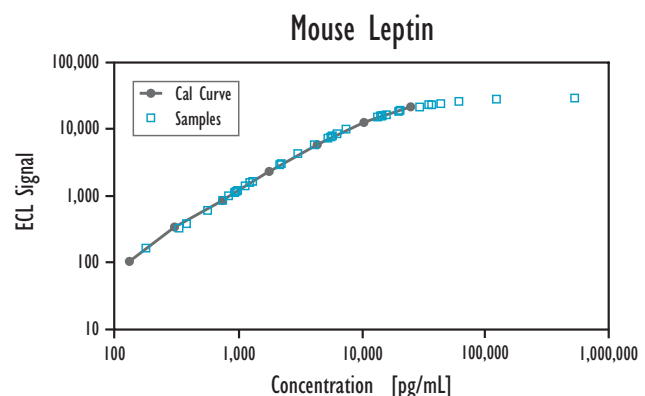
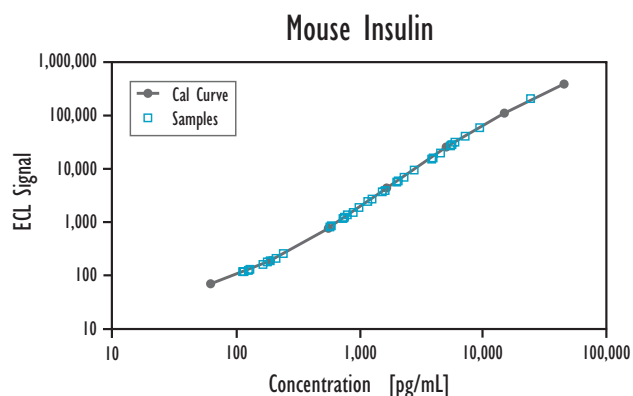
GLP-1 Levels in Fed Mice

Mice were exposed to food at time = 0 and a serum sample was collected from each mouse at a different time point for a total of 6 time points. The food was available throughout the duration of sample collection. These samples were probed for GLP-1 levels using the MSD GLP-1 assay. GLP-1 levels were expected to increase readily upon food consumption as this protein hormone plays a role in lowering blood glucose levels. The graph shows typical GLP-1 concentrations measured at each time point.



Insulin and Leptin Levels in Mice with *ob* and *db* Mutations

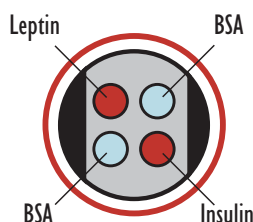
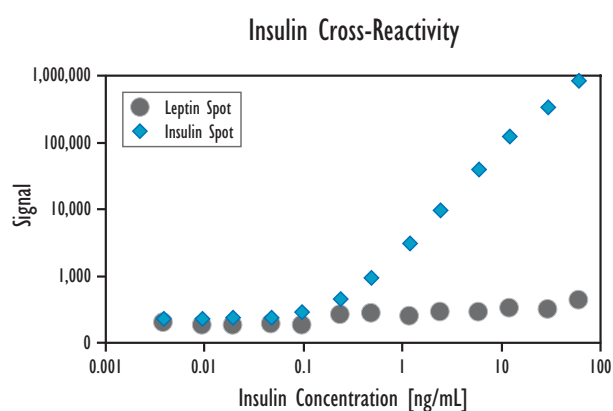
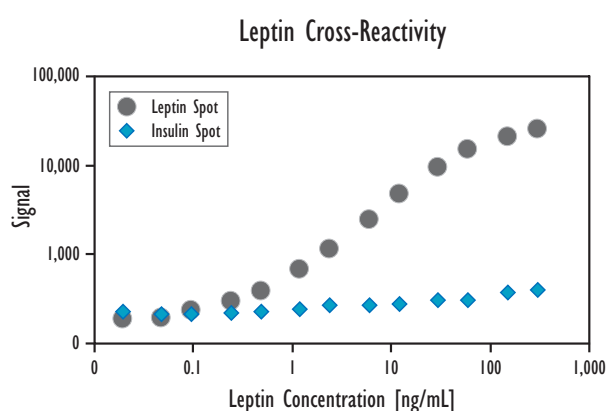
This study features samples with a large range of insulin and leptin levels. These samples were collected from mice possessing different combinations of *ob* or *db* mutations. The *ob* mutation causes an inactive form of leptin to be produced and the *db* gene causes a mutation in the leptin receptor making the brain insensitive to the action of circulating leptin. Note that leptin levels are expected to be high in mice exhibiting the *db* phenotype. The leptin calibration curve captures most of the native levels expressed in these samples, but a few of the endogeneous levels are so high in value that they need to be re-quantitated following sample dilution.



Applications of MSD Metabolic Marker Assays (continued)

Multiplexing Mouse Insulin and Leptin Assays

Mouse insulin and mouse leptin assays were multiplexed without any loss in assay performance. The measured cross-reactivity between the two assays is very low.



Mouse insulin and mouse leptin antibodies are coated on MSD MULTI-SPOT 4-spot plates for simultaneous measurement in the same well with minimal cross-reactivity.

Cross-Reactivity	Insulin Spot	Leptin Spot
Quantitated level in presence of 30 ng/mL insulin	29.6 ng/mL	0.3 ng/mL
Quantitated level in presence of 60 ng/mL leptin	0.1 ng/mL	63.4 ng/mL
Quantitated level in presence of 30 ng/mL insulin + 60 ng/mL leptin	29.6 ng/mL	66.0 ng/mL

Conclusions

- MSD metabolic marker assays are rapid (1-3 hrs) and nonradioactive.
- MSD metabolic marker assays exhibit a large dynamic range and good analytical sensitivity.
- MSD metabolic marker assays can be multiplexed into custom panels for simultaneous measurement of multiple analytes in the same well.