

High Throughput Assays for Mouse Metabolic Markers: Insulin, Leptin and Adiponectin

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1 Abstract

We have developed assays for mouse Insulin, Leptin and Adiponectin on the MSD MULTI-ARRAY™ platform for measurement in serum and plasma. All assays are sandwich immunoassays using electrochemiluminescent-labeled detection antibodies. The insulin and leptin assays are combined in 2-plex format for simultaneous measurement, and require only 5 μ L of sample. The adiponectin assay is carried out in a 1000-fold diluted sample, since its concentration is in the range of 3,000 – 15,000 ng/mL.

The assay protocols involve a single 2-hour incubation followed by a wash step. Assays are performed in MSD 96-well 4-spot or 1-spot plates and are read on MSD's SECTOR™ Imager 6000, at a throughput of 80 samples per minute. Assay ranges are 0.1 – 30 ng/mL (17 – 5,000 pM) for Insulin, 0.1 – 200 ng/mL (7 – 13,000 pM) for Leptin, and 0.17 – 200 μ g/mL (6 – 6,000 nM) for Adiponectin. All assays are highly specific and linear over the normal serum/plasma range for each analyte.

2 Background

INSULIN

- 5.8 kD hormone that elicits metabolic effects such as increase in glucose uptake and glycogen synthesis.
- Mouse insulin is 78% homologous to human insulin.
- Circulating levels of mouse insulin in serum are in the range 0.5 – 10 ng/mL.

LEPTIN

- 16 kD molecular weight. Mice that lack functional leptin are obese, diabetic, infertile, and have reduced activity, metabolism, and body temperature. Circulating levels of leptin are regulated in response to stimuli such as food intake, insulin, cytokines, glucocorticoids, and reproductive events.
- Mouse leptin is 84% homologous to human leptin.
- Circulating levels of leptin in mouse serum vary in the range 1 – 75 ng/mL and exhibit gender dichotomy.

ADIPONECTIN

- 30 kD molecular weight. Plays a role in lipid and glucose metabolism. Enhances insulin sensitivity by activating glucose uptake and accelerating fatty acid oxidation and clearance. Plays a role in immune response as well. May circulate as trimers, hexamers, or higher order multiples.
- Mouse adiponectin is 86% homologous to human adiponectin.
- Circulating levels of adiponectin in mouse serum are high: 3 – 15 μ g/mL.



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3 Insulin/Leptin Assay Components

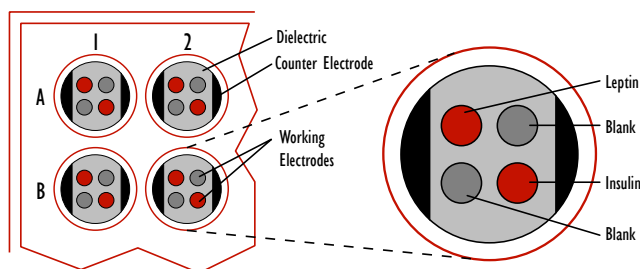
INSULIN/LEPTIN DUPLEX:

- Insulin/Leptin 2-Plex MSD Plate: 96-well 4-spot plate coated with antibodies against Insulin and Leptin.
- Detection Antibody Solution: A solution of electrochemiluminescent-labeled detection antibodies.
- Insulin/Leptin Standards: A stock solution of Insulin and Leptin to be used in the generation of the calibration curve.
- Assay Diluent: Animal serum-based solution for dilution of detection antibodies.
- Calibrator Diluent: A protein-based buffered solution with preservatives, for preparation of calibrators.

4 Protocol for Insulin/Leptin Duplex

- Add 45 μ L of Assay Diluent containing detection antibodies
- Add 5 μ L of sample (Rat or Mouse Serum/Plasma) or calibrator
- Incubate plate for 2 hours with shaking
- Wash with PBS
- Add MSD-T Read Buffer
- Read plate on SECTOR Imager 6000 Reader (~ 1 minute read time)

Total Assay Time is 2 hours



5 Adiponectin Assay Components

- Adiponectin MSD Plate: 96-well 1-spot plate coated with an antibody against Adiponectin.
- Detection Antibody Solution: A solution of electrochemiluminescent-labeled detection antibodies.
- Adiponectin Standards: A stock solution of Adiponectin standards to be used in the generation of the calibration curve after serial dilution.
- Assay Diluent: A protein-based buffered solution with preservatives for dilution of calibrators and detection antibodies.



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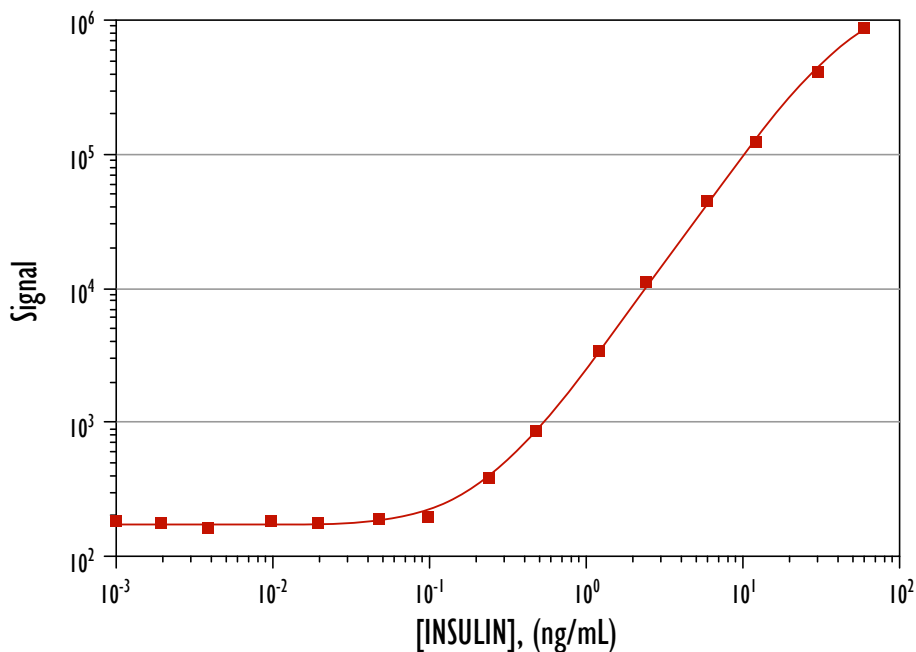
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6 Protocol for Adiponectin

- Block with MSD Blocker A for 1 - 2 hours at room temperature or overnight at 4° C
- Wash with PBS
- Add 40 μ L of Assay Diluent containing detection antibodies
- Add 10 μ L of 1000-fold diluted sample (Mouse Serum/Plasma) or calibrator, diluted with Assay Diluent
- Incubate plate for 2 hours with shaking
- Wash with PBS
- Add MSD-T Read Buffer
- Read plate on SECTOR Imager 6000 Reader (1 min read time)

Total Assay Time 2 – 4 hours.

7 Insulin Calibration Curve



Measurement Range: 0.1 – 30 ng/mL (5 μ L Sample)
Detection Limit: 0.1 ng/mL

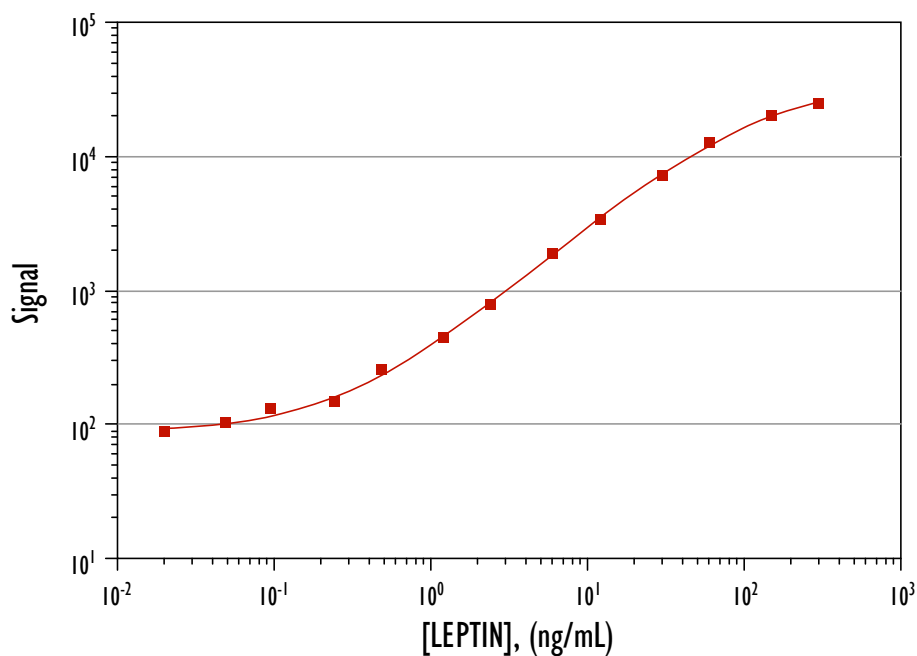
Curve Represents 4-parameter fit: $y = b_1 + \frac{b_2 - b_1}{1 + (x/b_3)^{b_4}}$



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8 Leptin Calibration Curve



Measurement Range: 0.1 – 300 ng/mL (5 μ L Sample)

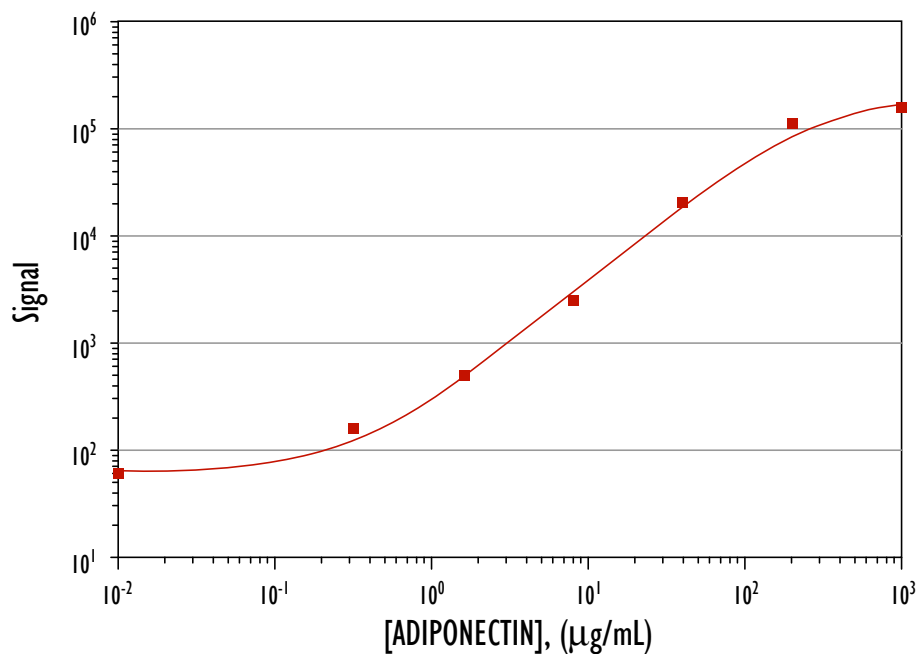
Detection Limit: 0.1 ng/mL



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9 Adiponectin Calibration Curve



Measurement Range: 0.17 – 200 $\mu\text{g/mL}$ (10 nL sample per well)

Detection Limit: 0.17 $\mu\text{g/mL}$



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10 Expected Values and Spike/Recovery

Native levels and recovery were calculated based on 17 rat and 18 mouse samples.

Samples included individual plasma (EDTA and Li-heparin) and serum samples, and pooled male and female serum. Spike levels were 3 ng/mL for Insulin, 10 ng/mL for Leptin and 100 µg/mL for Adiponectin.

INSULIN	RAT	MOUSE
Average Native Level, ng/mL	0.43	0.8
Range of Native Level, ng/mL	0.06 - 1.89	0.1 - 2.9
Average Recovery	86%	92%

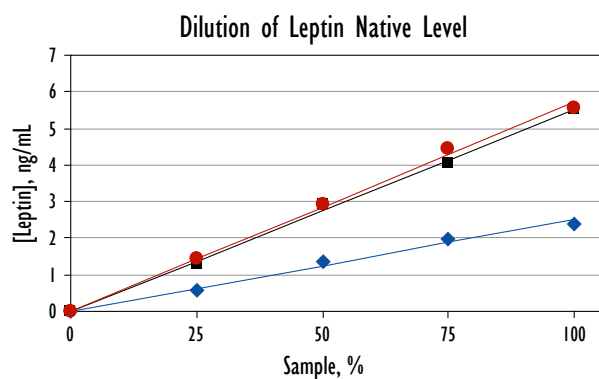
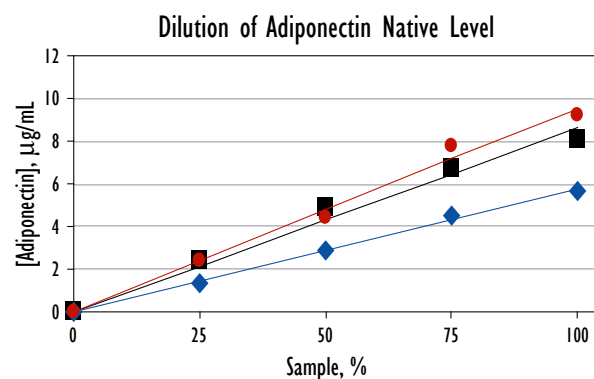
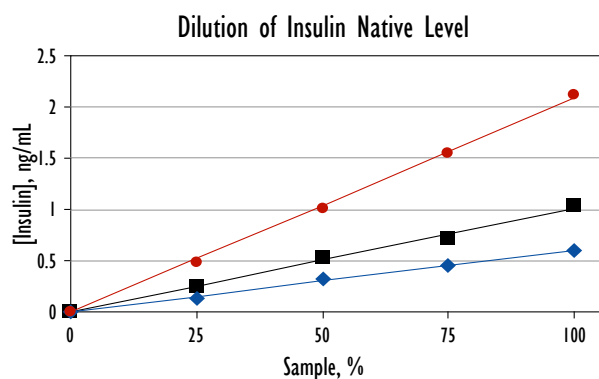
LEPTIN	RAT	MOUSE
Average Native Level, ng/mL	To Be Developed	3.6
Range of Native Level, ng/mL		1.5 - 6.6
Average Recovery		98%

ADIPONECTIN	RAT	MOUSE
Average Native Level, µg/mL	To Be Developed	15
Range of Native Level, µg/mL		9.7 - 37
Average Recovery		107%



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II Dilution Series of Serum and Plasma Samples: Native Levels



Three mouse serum/plasma samples were diluted with calibrator diluent and analyzed for Insulin, Leptin and Adiponectin content.

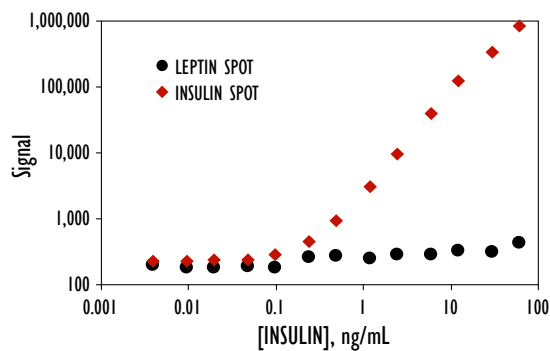
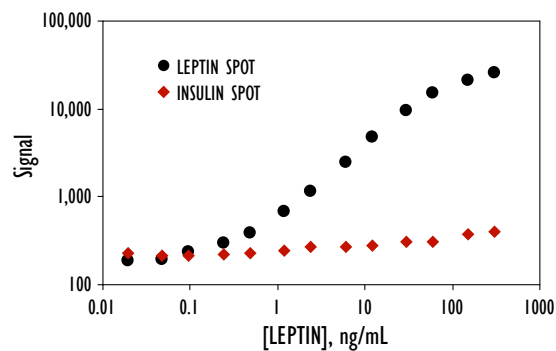
All three analytes dilute linearly



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12 Multiplexing: Specificity/Cross-Reactivity



	INSULIN SPOT	LEPTIN SPOT
Background Signal	232	196
Signal at 30 ng/mL Insulin	340,288	319
Signal at 60 ng/mL Leptin	310	15,225
Signal at 30 ng/mL Insulin + 60 ng/mL Leptin	340,089	15,595
Cross-Reactivity	0.5%	0.04%

Cross-Reactivity is below 0.5 %



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13 MSD Assays versus ELISA Kits

Assay Parameters	MSD	ELISA
INSULIN		
Detection Limit	0.1 ng/mL	0.2 ng/mL
Sample Size	5 μ L	10 μ L
Time to Result	2 hours	3 hours
Number of Wash Steps	1	3
LEPTIN		
Detection Limit	0.1 ng/mL	0.02 ng/mL
Sample Size	5 μ L	100 μ L
Time to Result	2 hours	3 hours
Number of Wash Steps	1	3
ADIPONECTIN		
Detection Limit	0.17 μ g/mL	0.2 μ g/mL
Sample Size	10 nL	10 nL
Time to Result	2 hours	>3 hours
Number of Wash Steps	1	4

The MSD Assays provide similar or lower detection limits compared to commercial ELISA kits, while using smaller sample sizes, shorter incubation times and fewer number of wash steps.

Multiplexing on the MSD MULTI-ARRAY plates allows measurement of Insulin and Leptin simultaneously in one 5 μ L sample.

14 Conclusions

- MSD MULTI-ARRAY assays have been developed for Mouse/Rat Insulin, Leptin and Mouse Adiponectin.
- Insulin and Leptin assays are available in multiplexed format.
- Assay features include:
 - Detection limits for Insulin, Leptin and Adiponectin well below native levels and comparable to available ELISA kits.
 - Simple protocols and low sample volumes (5 μ L of undiluted samples for Insulin/Leptin and 1/1000 dilution for Adiponectin).
 - Dynamic ranges that extend well beyond the clinical ranges.
 - Precision (CV) of less than 10%.
 - Good recoveries and dilution linearity in serum, Li-Heparin and EDTA plasma.
 - Very low cross-reactivity in the multiplexed Insulin/Leptin assays.
 - Insensitivity towards anti-coagulants.

