

Meso Scale Discovery[®]

MULTI-ARRAY[®] Assay System

Rat Adiponectin Kit

1-Plate Kit

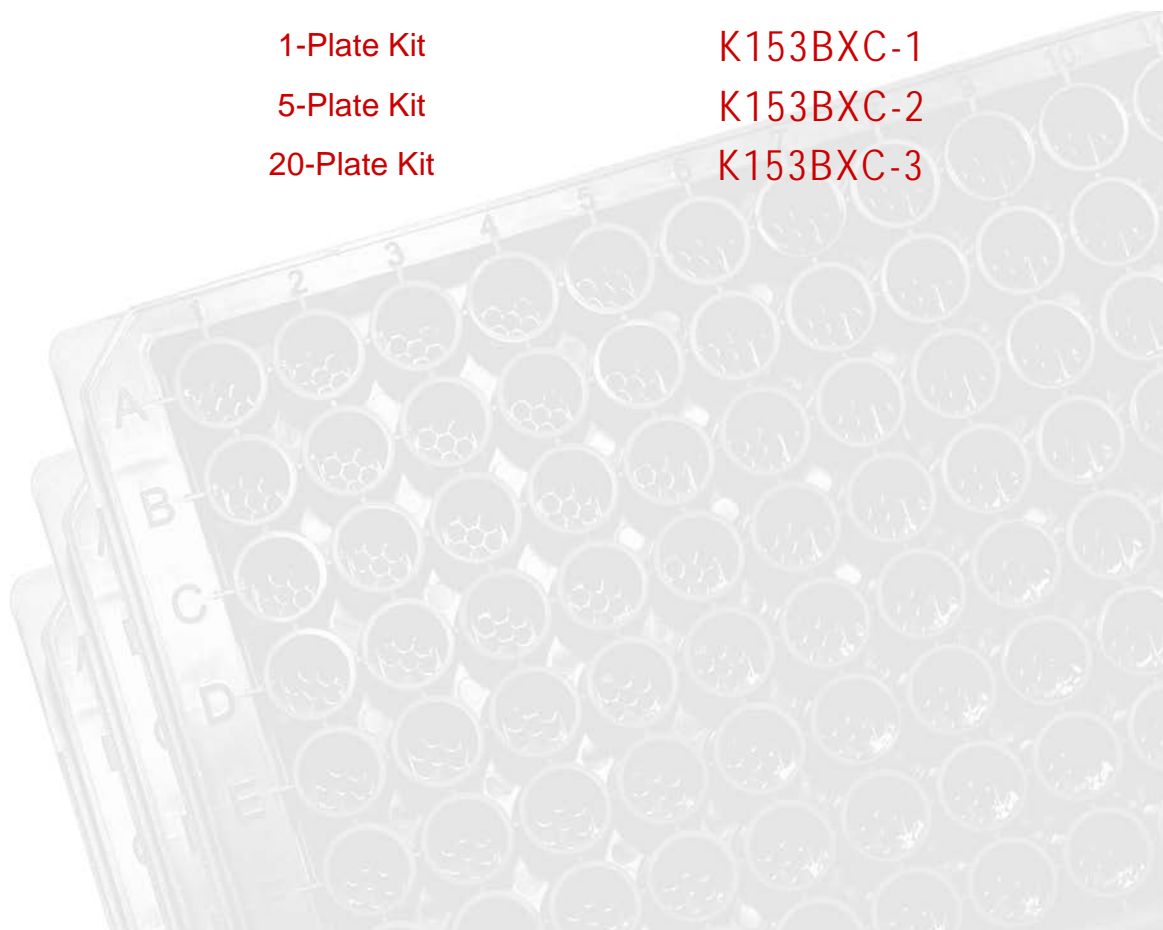
K153BXC-1

5-Plate Kit

K153BXC-2

20-Plate Kit

K153BXC-3



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MSD Metabolic Assays

Rat Adiponectin Kit

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Meso Scale Discovery

A division of Meso Scale Diagnostics, LLC.

9238 Gaither Road

Gaithersburg, MD 20877 USA

www.mesoscale.com

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Ordering Information

ordering information

MSD Customer Service

Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Scientific Support

Phone: 1-301-947-2025
Fax: 1-240-632-2219 attn: Scientific Support
Email: ScientificSupport@mesoscale.com

Introduction

introduction

Adiponectin is a 30 kD protein secreted exclusively by adipocytes and is thought to play a role in lipid and glucose metabolism. Adiponectin enhances insulin action by activating glucose uptake and fatty acid oxidation. In addition, this adipokine has potent anti-inflammatory and anti-atherosclerotic properties. Adiponectin circulates at high levels (between 2-20 $\mu\text{g/mL}$) in plasma as trimers, hexamers and high molecular weight multimers. In contrast to most adipokines, plasma adiponectin is often negatively correlated with body mass index (BMI) in humans and rodents. However, expression in various adipose tissues and disease models may vary.

Principle of the Assay

principle of the assay

MSD[®] metabolic assays provide rapid and convenient methods for measuring the levels of protein targets within single small-volume samples. The assays are available in both singleplex and multiplex formats. In a singleplex assay, an antibody for a specific protein target is coated on one electrode (or “spot”) per well. In a multiplex assay, an array of capture antibodies against different targets is patterned on distinct spots in the same well. Our Rat Adiponectin Assay detects adiponectin in a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with adiponectin antibody. The user adds the sample and a solution containing the labeled detection antibody—anti-adiponectin labeled with an electrochemiluminescent compound, MSD SULFO-TAG[™] label—over the course of one or more incubation periods. Adiponectin in the sample binds to capture antibody immobilized on the working electrode surface; recruitment of the labeled detection antibody by bound analyte completes the sandwich. The user adds an MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD SECTOR instrument for analysis. Inside the SECTOR instrument, a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. The instrument measures intensity of emitted light to afford a quantitative measure of adiponectin present in the sample.

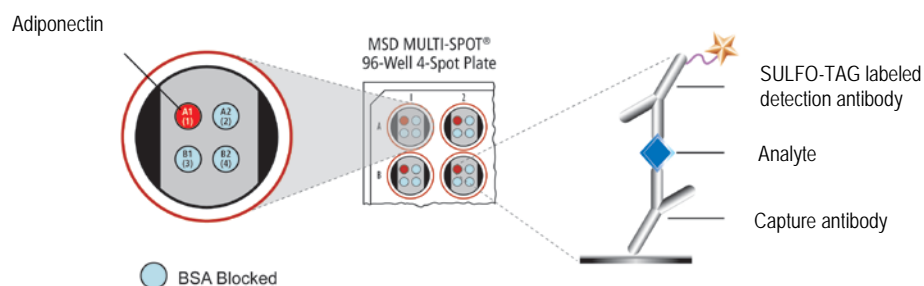


Figure 1. Sandwich immunoassay on MSD platform. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. Any spot that is not coated with a specific capture antibody is blocked with BSA to reduce non-specific binding to that spot. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.



Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit		
		K153BXC-1	K153BXC-2	K153BXC-3
MULTI-SPOT 96-well Rat Adiponectin Plate(s) N453BXA-1	2-8°C	1 plate	5 plates	20 plates
SULFO-TAG Anti-rAdiponectin Antibody ¹ (100X)	2-8°C	1 vial (40 µL)	1 vial (200 µL)	4 vials (200 µL ea)
Rat Adiponectin Calibrator 10 µg/mL	≤-70°C	1 vial (20 µL)	5 vials (20 µL ea)	20 vials (20 µL ea)
Blocker A Kit R93AA-2 (250 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	4 bottles (250 mL ea)
Diluent 100 R50AA-2 (200 mL) R50AA-3 (1 L)	2-8°C	1 bottle (200 mL)	2 bottles (200 mL ea)	2 bottles (200 mL & 1 L)
Read Buffer T (4X) R92TC-3 (50 mL) R92TC-2 (200 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	1 bottle (200 mL)



Required Materials and Equipment - not supplied

required materials and equipment — not supplied

- Deionized water for diluting concentrated buffers
- 50 mL tubes for reagent preparation
- 15 mL tubes for reagent preparation
- Microcentrifuge tubes for preparing serial dilutions
- Phosphate buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- Appropriate liquid handling equipment for desired throughput, capable of dispensing 25 to 150 µL into a 96-well microtiter plate
- Plate washing equipment: automated plate washer or multichannel pipette
- Adhesive plate seals
- Microtiter plate shaker



Safety

safety

Safe laboratory practices and personal protective equipment such as gloves, safety glasses, and lab coats should be used at all times during the handling of all kit components. All hazardous samples should be handled and disposed of properly, in accordance with local, state, and federal guidelines.

¹ Some SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

VI Reagent Preparation

reagent preparation

Bring all reagents to room temperature and thaw the Calibrator stock on ice.

Prepare Blocker A Solution

Follow instructions included with the Blocker A Kit.

Prepare Calibrator and Control Solutions

Calibrator for the Rat Adiponectin Assay is supplied at 10 µg/mL. For the assay, an 8-point standard curve is recommended with 3-fold serial dilution steps and a zero Calibrator. The table below shows the concentrations of the 8-point standard curve:

Standard	Adiponectin conc. (ng/mL)	Dilution Factor
Stock Cal. Vial	10000	
STD-01	200	50
STD-02	67	3
STD-03	22	3
STD-04	7.4	3
STD-05	2.5	3
STD-06	0.82	3
STD-07	0.27	3
STD-08	0	n/a

To prepare this 8-point standard curve:

- 1) Prepare the highest Calibrator by transferring 10 µL of the Calibrator stock vial to 490 µL of Diluent 100.
- 2) Prepare the next Calibrator by transferring 100 µL of the diluted Calibrator to 200 µL of Diluent 100. Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.
- 3) The recommended 8th Standard is Diluent 100 (i.e. zero Calibrator).
- 4) Diluted Calibrators should be kept on ice prior to addition to the plate.

Note: The standard curve can be modified as necessary to meet specific assay requirements.

Preparation of Serum and Plasma Samples

The assay format requires 10 µL of pre-diluted serum/plasma samples per well. An adequate volume of each sample should be prepared depending upon desired number of replicates. The serum/plasma samples should be pre-diluted with Diluent 100 one thousand fold (some samples may require larger dilutions). A two-step dilution is recommended; an initial 10-fold dilution followed by a second 100-fold dilution.

- 10 µL of sample + 90 µL of Diluent 100
- 10 µL of the above dilution + 990 µL of Diluent 100

Prepare Detection Antibody Solution

The Detection Antibody is provided at 100X stock solution. The final concentration of the working Detection Antibody Solution should be at 1X. For each plate used, dilute a 30 µL aliquot of the stock Detection Antibody into 2.97 mL of Diluent 100.

Prepare Read Buffer

The Read Buffer should be diluted 4-fold in deionized water to make a final concentration of 1X Read Buffer T. Add 5 mL of 4X Read Buffer T to 15 mL of deionized water for each plate.

Prepare MSD Plate

This plate has been pre-coated with antibody for the analyte shown in Figure 1. The plate can be used as delivered; no additional preparation (e.g., pre-wetting) is required. The plate has also been exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies.

VII Assay Protocol

assay protocol

- 1. Addition of Blocker A Solution:** Dispense 150 μL of Blocker A Solution into each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.
- 2. Wash and Addition of Sample or Calibrator:** Wash the plate 3 times with PBS-T. Dispense 40 μL of Diluent 100 into each well of the MSD plate. Immediately add 10 μL of sample or Calibrator into the appropriate wells of the MSD plate. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
- 3. Wash and Addition of the Detection Antibody Solution:** Wash the plate 3 times with PBS-T. Dispense 25 μL of the 1X Detection Antibody Solution into each well of the MSD plate. Seal the plate and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.
- 4. Wash and Read:** Wash the plate 3 times with PBS-T. Add 150 μL of 1X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. Plates may be read immediately after the addition of Read Buffer.

Notes

Shaking a 96-well MSD MULTI-SPOT plate typically accelerates capture at the working electrode.

Bubbles in the fluid will interfere with reliable reading of MULTI-SPOT plate. Use reverse pipetting techniques to insure bubbles are not created when dispensing the Read Buffer.

VIII Analysis of Results

analysis of results

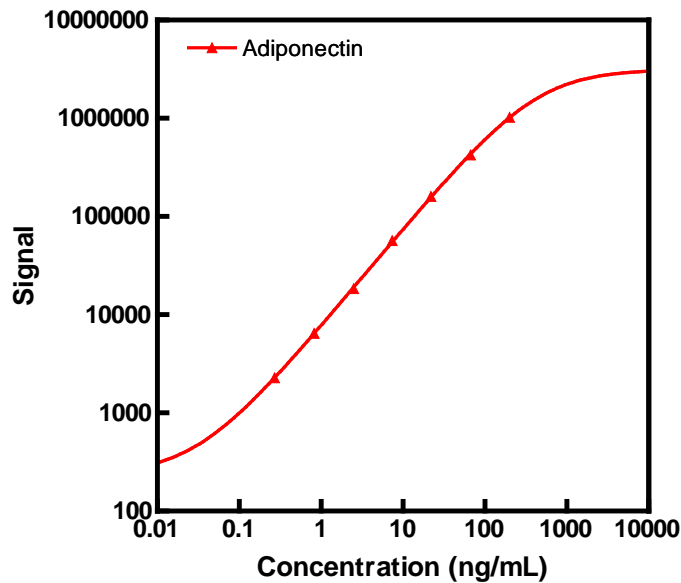
The Calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have a wide dynamic range (3–4 logs) which allows accurate quantification in many samples without the need for dilution. The MSD DISCOVERY WORKBENCH[®] analysis software utilizes a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting functionality is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

IX Typical Standard Curve

typical standard curve

The MSD Rat Adiponectin Assay is designed for use with rat serum and plasma samples.

The following standard curve is an example of the dynamic range of the assay. The actual signals may vary. A standard curve should be run for each set of samples and on each plate for the best quantification of unknown samples.



Adiponectin		
Conc. (ng/mL)	Average Signal	%CV
0	229	11.7
0.27	2282	9.7
0.82	6467	11.1
2.5	18472	10.0
7.4	56694	1.8
22	159706	7.2
67	422586	3.7
200	1024238	5.0

X Sensitivity

sensitivity

The lower limit of detection (LLOD) is the calculated concentration of the signal that is 2.5 standard deviations over the zero Calibrator. The value below represents the average LLOD over multiple kit lots.

Adiponectin	
LLOD (ng/mL)	0.11

XI

Endogenous Levels

endogenous levels

Endogenous levels of rat adiponectin in five matched individual serum and plasma samples. Samples were diluted 1000 fold prior to measurement. The data below represents final concentrations which have been corrected for dilutions.

Sample	Serum (µg/mL)	EDTA Plasma (µg/mL)	Heparin Plasma (µg/mL)
1	12	7	12
2	11	5	8
3	25	13	19
4	6	3	5
5	8	3	6

XII

Spike Recovery

spike recovery

Diluted serum, EDTA plasma, and heparin plasma were spiked with the Calibrators at multiple values throughout the range of the assay. Measured analyte represents average spike recovery in pooled rat samples.

% Recovery = measured /expected x 100

	Spike Conc. (µg/mL)	% Recovery
Spiked Serum	5	103
	50	98
	500	90
Spiked EDTA Plasma	5	100
	50	102
	500	106
Spiked Heparin Plasma	5	100
	50	101
	500	102

XIII Linearity

linearity

Linearity was measured by spiking Calibrator levels in pooled rat samples followed by subsequent dilution.

Percent recovery is calculated as the measured concentration divided by the concentration of the previous dilution (expected).

$$\% \text{ Recovery} = \text{measured} \times \text{dilution factor} / \text{expected} \times 100$$

	Fold Dilution	% Recovery
Serum	2	102
	4	102
	8	101
EDTA Plasma	2	97
	4	98
	8	94
Heparin Plasma	2	100
	4	96
	8	97

XIV Assay Components

assay components

Calibrator	
Analyte	Rat adiponectin
Source	Purified, recombinant rat adiponectin with a FLAG-Tag expressed in HEK 293

Capture Antibody	
Analyte	Rat adiponectin
Source	Goat polyclonal
Isoforms Recognized	n/a
Species cross-reactivity	Rat adiponectin, shares 91% and 83% amino acid sequence homology with mouse and human adiponectin respectively

Detection Antibody	
Analyte	Rat adiponectin
Source	Goat polyclonal
Isoforms Recognized	n/a
Species cross-reactivity	Rat adiponectin, shares 91% and 83% amino acid sequence homology with mouse and human adiponectin respectively

1. Lihn AS, Pedersen SB, Richelsen B. Adiponectin: action, regulation and association to insulin sensitivity. *Obesity Reviews*. 2005 Feb;6(1):13-21
2. Tsao TS, Murry HE, Hug C et al.. Oligomerization state-dependent activation of NF-kappa B signaling pathway by adipocyte complement-related protein of 30 kDa (Acrp30). 2002 *J. Biol. Chem.* Aug;277(33): 29359-62
3. Combs TP, Pajvani UB, Berg AH et al. A transgenic mouse with a deletion in the collagenous domain of adiponectin displays elevated circulating adiponectin and improved insulin sensitivity. *Endocrinology*. 2004 Jan;145(1)::367-83
4. Fruebis J, Tsao TS, Jauorschi S et al. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. 2001 *PNAS* Feb;98(4): 2005-10
5. Fasshauer M, Paschke R, Stumvoll M. Adiponectin, obesity, and cardiovascular disease. 2004 *Biochimie* Nov;86(11):779-86

Summary Protocol
MSD 96-well MULTI-ARRAY Rat Adiponectin Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the Rat Adiponectin Assay.

Step 1 : Sample and Reagent Preparation

Bring all reagents to room temperature and thaw the Calibrator stock on ice.

Prepare Blocker A Solution.

Prepare serum or plasma samples.

Prepare an 8-point standard curve using supplied Calibrator:

- The Calibrator should be diluted in Diluent 100.
- Dilute the stock Calibrator 1:50 in Diluent 100 then perform a series of 3-fold dilution steps and a no Calibrator blank.
- Diluted Calibrators should be kept on ice until use.

Note: The standard curve can be modified as necessary to meet specific assay requirements.

Prepare Detection Antibody Solution by diluting the 100X Anti-rAdiponectin Antibody to 1X in 3.0 mL of Diluent 100 per plate.

Prepare 20 mL of 1X Read Buffer T by diluting 4X Read Buffer T with deionized water.

Step 2 : Add Blocker A Solution

Dispense 150 μ L/well Blocker A Solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 3 : Wash and Add Sample or Calibrator

Wash plate 3 times with PBS-T.

Dispense 40 μ L/well Diluent 100.

Immediately, dispense 10 μ L/well Calibrator or Sample.

- Samples should be diluted 1000-fold as described in the Reagent Preparation section.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 4 : Wash and Add Detection Antibody Solution

Wash plate 3 times with PBS-T.

Dispense 25 μ L/well 1X Detection Antibody Solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 5 : Wash and Read Plate

Wash plate 3 times with PBS-T.

Dispense 150 μ L/well 1X Read Buffer T.

Analyze plate on SECTOR instrument.

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