

Meso Scale Discovery[®]

MULTI-ARRAY[®] Assay System

Mouse/Rat Resistin Kit

1-Plate Kit

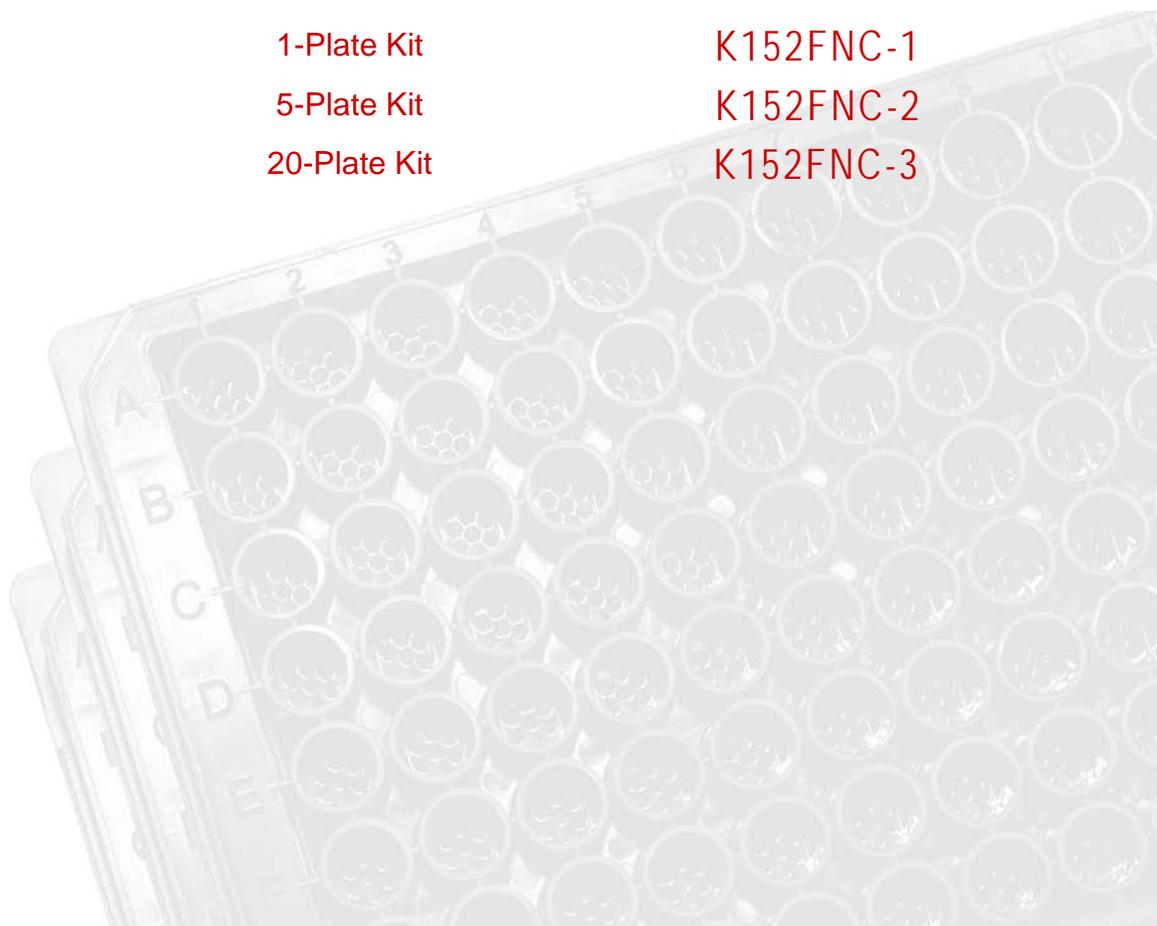
K152FNC-1

5-Plate Kit

K152FNC-2

20-Plate Kit

K152FNC-3



Meso Scale Discovery Meso Scale Di



MSD Metabolic Assays

Mouse/Rat Resistin 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

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Ordering Information

ordering information

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Introduction

introduction

Resistin is a 12.5 kDa peptide hormone, derived from adipocytes and immune cells, whose physiologic role has been the subject of much controversy regarding its involvement with obesity and type II diabetes mellitus (T2DM). In mice, administration of anti-resistin antibody improves blood sugar and insulin action. Moreover, treatment of normal mice with recombinant resistin impairs glucose tolerance and insulin action. In addition, serum resistin levels are elevated in two different genetic models (*ob/ob* and *db/db*) as in a diet-induced model of diabetes. Thus, resistin might link obesity with insulin resistance and diabetes in murine models.

Expression of resistin in human adipocytes is very low compared with that seen in rodents and does not differ between normal, insulin-resistant and T2DM individuals. In humans, resistin is expressed in inflammatory cells, leukocytes, and macrophages and has the potency of inducing production of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α). Similarly, resistin is accumulated in inflamed joints of patients with RA and has the capacity to induce arthritis in mice. Taken together, resistin may therefore play a more vital role in the well-establish association between inflammation and insulin resistance.

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 Mouse/Rat Resistin Assay detects resistin in a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with resistin antibody. The user adds the sample and a solution containing the labeled detection antibody—anti-resistin labeled with an electrochemiluminescent compound, MSD SULFO-TAG[™] label—over the course of one or more incubation periods. Resistin 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 resistin present in the sample.

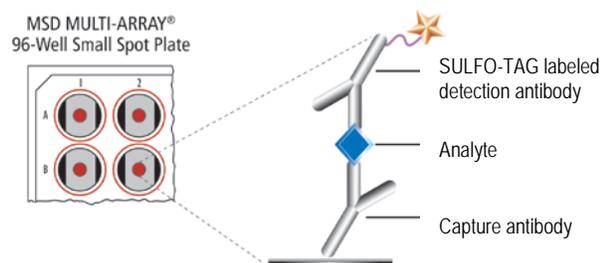


Figure 1. Sandwich immunoassay on MSD platform



Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit		
		K152FNC-1	K152FNC-2	K152FNC-3
MULTI-ARRAY 96-well Mouse/Rat Resistin Plate(s) L452FNA-1 (K152FNC)	2-8°C	1 plate	5 plates	20 plates
SULFO-TAG Anti-m/r Resistin Antibody ¹ (100X)	2-8°C	1 vial (40 µL)	1 vial (200 µL)	4 vials (200 µL ea)
Mouse/Rat Resistin 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 18 R50FA-3 (50 mL)	≤-10°C	1 bottle (50 mL)	1 bottle (50 mL)	4 bottles (50 mL ea)
Diluent 11 R55BA-3 (50 mL)	≤-10°C	1 bottle (50 mL)	1 bottle (50 mL)	4 bottles (50 mL ea)
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 Mouse/Rat Resistin 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	Resistin conc. (pg/mL)	Dilution Factor
Stock Cal. Vial	10000000	
Diluted Cal. Vial	100000	100
STD-01	12500	8
STD-02	4167	3
STD-03	1389	3
STD-04	463	3
STD-05	154	3
STD-06	51	3
STD-07	17	3
STD-08	0	n/a

To prepare this 8-point standard curve:

- 1) Dilute the Mouse/Rat Resistin Calibrator to a concentration of 0.1 µg/mL. Add 10 µL of the Calibrator stock solution to 990 µL of Diluent 11.
- 2) Prepare the highest Calibrator by transferring 50 µL of the diluted Calibrator solution at 0.1 µg/mL to 350 µL of Diluent 11.
- 3) Prepare the next Calibrator by transferring 100 µL of the diluted Calibrator to 200 µL of Diluent 11. Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.
- 4) The recommended 8th Standard is Diluent 11 (i.e. zero Calibrator).
- 5) 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 11 twenty-fold. In order to ensure accuracy, pipetting volumes less than 5 µL should be avoided while performing dilutions. A smaller dilution factor (5-fold) is recommended for rat samples.

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 11.

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 18 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-ARRAY plate typically accelerates capture at the working electrode.

Bubbles in the fluid will interfere with reliable reading of MULTI-ARRAY 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.

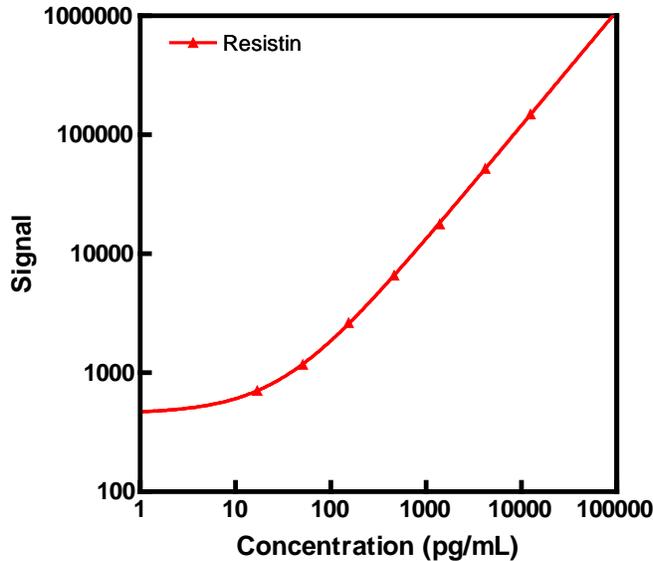
IX

Typical Standard Curve

typical standard curve

The MSD Mouse/Rat Resistin Assay is designed for use with mouse 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.



Resistin		
Conc. (pg/mL)	Average Signal	%CV
0	428	3.1
17	707	7.2
51	1174	7.9
154	2642	5.6
463	6593	4.3
1389	17753	9.3
4167	52023	4.9
12500	149506	5.1

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.

Resistin	
LLOD (pg/mL)	1.9

XI

Endogenous Levels

endogenous levels

Endogenous levels of resistin in five matched individual serum and plasma samples. The data below represents final concentrations which have been corrected for dilutions.

Endogenous resistin levels in mouse samples

Mouse samples were diluted 20 fold prior to measurement.

Sample	Serum (ng/mL)
1	8.6
2	11
3	5.2
4	9.6
5	5.7
6	14
7	8.2
8	23

Endogenous resistin levels in rat samples

Rat samples were diluted 5 fold prior to measurement.

Sample	Serum (ng/mL)	EDTA Plasma (ng/mL)	Heparin Plasma (ng/mL)
1	6.6	5.9	8.0
2	8.1	8.5	9.2
3	13	10	15
4	6.1	5.1	6.5
5	3.6	3.6	5.3

XII Spike Recovery

spike recovery

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

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

	Spike Conc. (µg/mL)	% Recovery	
		Mouse	Rat
Spiked Serum	5	96	105
	50	100	101
	500	110	112
Spiked EDTA Plasma	5	101	104
	50	116	96
	500	123	103
Spiked Heparin Plasma	5	91	104
	50	88	96
	500	98	90

XIII Linearity

linearity

Linearity was measured by spiking Calibrator levels in pooled mouse and 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	
		Mouse	Rat
Serum	2	93	108
	4	92	112
	8	96	126
EDTA Plasma	2	91	110
	4	88	112
	8	98	128
Heparin Plasma	2	91	105
	4	90	105
	8	95	108

XIV

Assay Components

assay components

Calibrator	
Analyte	Mouse resistin
Source	Purified, recombinant murine resistin expressed in E. Coli

Capture Antibody	
Analyte	Mouse resistin
Source	goat polyclonal
Isoforms Recognized	n/a
Species cross-reactivity	Mouse, rat

Detection Antibody	
Analyte	Mouse/Rat resistin
Source	Goat polyclonal
Isoforms Recognized	n/a
Species cross-reactivity	Mouse, not human

XV

References

references

1. Stepan CM, Lazar MA. The current biology of resistin. *J Intern Med.* 2004 Apr;255(4):439-47
2. Stepan CM, Brown EJ, Wright CM, et al. A family of tissue-specific resistin-like molecules. *Proc Natl Acad Sci U S A.* 2001 Jan 16;98(2):502-6
3. Rajala MW, Qi Y, Patel HR, et al. Regulation of resistin expression and circulating levels in obesity, diabetes, and fasting. *Diabetes.* 2004 Jul;53(7):1671-9
4. Gerber M, Boettner A, Seidel B, et al. Serum resistin levels of obese and lean children and adolescents: biochemical analysis and clinical relevance. *J Clin Endocrinol Metab.* 2005 Aug;90(8):4503-9

Summary Protocol

MSD 96-well MULTI-ARRAY Mouse/Rat Resistin Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the Mouse/Rat Resistin 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 11.
- Dilute the stock Calibrator 1:800 in Diluent 11 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-m/r Resistin Antibody to 1X in 3.0 mL of Diluent 11 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 18.

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

- Samples should be diluted 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.

	1	2	3	4	5	6	7	8	9	10	11	12
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