MSD MULTI-ARRAY® Assay System

Human RBP4 Kit

1-Plate Kit	K151LXD-1
5-Plate Kit	K151LXD-2
25-Plate Kit	K151LXD-4

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MSD® Toxicology Assays

Human RBP4 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

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

Retinol-binding protein 4 (RBP4) is a 21 kDa member of the lipocalin superfamily that transports Vitamin A (retinol) from liver stores to peripheral tissues via serum. The RBP4-retinol complex interacts with transthyretin (TTR) and prevents it from being filtered by the kidney.[1] The C-terminal, processed forms of RBP4 do not bind TTR; they are excreted into the urine and, during renal failure, they accumulate in the serum.[2]

RBP4 also acts as an adipokine and has been linked to the development of obesity, type 2 Diabetes (T2DM) and insulin resistance. The protein is secreted by adipocytes and hepatocytes and promotes hyperglycemia through downregulation of the glucose transporter type 4 (GLUT4).[3] Glucose transport via Glut4 is the rate-limiting step for glucose use by muscle and adipose tissue. These processes are impaired in adipocytes of obese individuals and those with T2DM. Elevated RBP4 in urine and serum often mirrors the onset of cardiovascular complications and acute renal dysfunction associated with these diseases.[2,3] Thus, measurement of urine, serum, or plasma RBP4 is a useful means for the understanding of various metabolic disorders.

Principle of the Assay

MSD toxicology assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The Human RBP4 assay is a sandwich immunoassay (Figure 1). MSD provides a plate pre-coated with capture antibodies. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into a SECTOR® Imager where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures intensity of emitted light to provide a quantitative measure of analytes in the sample.

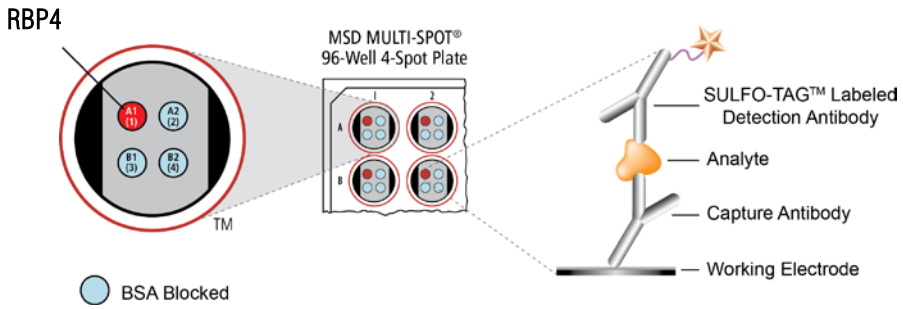


Figure 1. Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

Reagents Supplied

Product Description	Storage	Quantity per Kit K151LXD-1	K151LXD-2	K151LXD-4
MULTI-SPOT 96-Well 4-Spot Human RBP4 Plate N451LXA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu RBP4 Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human RBP4 Calibrator (1 µg/mL)	≤-70°C	1 vial (20 µL)	5 vials (20 µL ea)	25 vials (20 µL ea)
Diluent 37 R50AF-3 (25 mL) R50AF-6 (125 mL)	≤-10°C	2 bottles (25 mL)	2 bottles (125 mL ea)	10 bottles (125 mL ea)
Blocker A Kit R93AA-2 (250 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	5 bottles (250 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

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 10 to 150 µL into a 96-well microtiter plate
- Plate washing equipment: automated plate washer or multichannel pipette
- Adhesive plate seals
- Microtiter plate shaker

[1] SULFO-TAG conjugated detection antibodies should be stored in the dark.

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Reagent Preparation

Bring all reagents to room temperature. Thaw the stock calibrator on ice.

Important: Upon first thaw, separate Diluent 37 into aliquots appropriate to the size of your assay needs.

Prepare Blocker A Solution

Follow instructions included with the Blocker A Kit.

Prepare Standards

MSD recommends an 8-point standard curve with 4-fold serial dilution steps and a zero calibrator. Thaw the stock calibrator and keep on ice, then add to diluent at room temperature to make the standard curve solutions.

Standard	RBP4 Calibrator (pg/mL)	Dilution Factor
Stock Calibrator	1 000 000	
STD-01	50 000	20
STD-02	12 500	4
STD-03	3125	4
STD-04	781	4
STD-05	195	4
STD-06	49	4
STD-07	12	4
STD-08	0	n/a

To prepare 8 standard solutions for up to 3 replicates:

1) Prepare the highest standard by adding 15 µL of the calibrator stock to 285 µL of Diluent 37. Mix well.
2) Prepare the next standard by transferring 75 µL of the highest standard to 225 µL of Diluent 37. Mix well. Repeat 4-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 37 as the 8th standard (i.e. zero calibrator).

Dilute Samples

For urine samples, MSD recommends a 50-fold dilution in Diluent 37; however, you may adjust dilution factors for the sample set under investigation.

Prepare Detection Antibody Solution

MSD provides detection antibody in a 50X stock solution. The working detection antibody solution is 1X.

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-hu RBP4 Antibody
- ❑ 2.94 mL of Diluent 37

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 2X.

For 1 plate, combine:

- ❑ 10 mL Read Buffer T (4X)
- ❑ 10 mL deionized water

You may prepare diluted read buffer in advance and store it at room temperature in a tightly sealed container.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (see Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates can be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Assay Protocol

Notes

1. **Add Blocker A Solution:** Add 150 µL of Blocker A solution to each well. Seal the plate with an adhesive plate seal, and incubate for 30 minutes with vigorous shaking (300–1000 rpm) at room temperature.

Shaking the plate typically accelerates capture at the working electrode.

2. **Wash and Add Sample or Calibrator:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 50 µL of calibrator or diluted sample per well. Seal the plate with an adhesive plate seal, and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.

   You may prepare detection antibody solution during incubation.

3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 25 µL of 1X detection antibody solution into each well 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.

   You may prepare diluted read buffer during incubation.

You may keep excess diluted read buffer in a tightly sealed container at room temperature for later use.

4. **Wash and Read:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 150 µL of 2X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. No incubation in read buffer is required.

Bubbles introduced when adding read buffer will interfere with imaging of the plate and produce unreliable data. Use reverse pipetting technique to avoid creating bubbles.

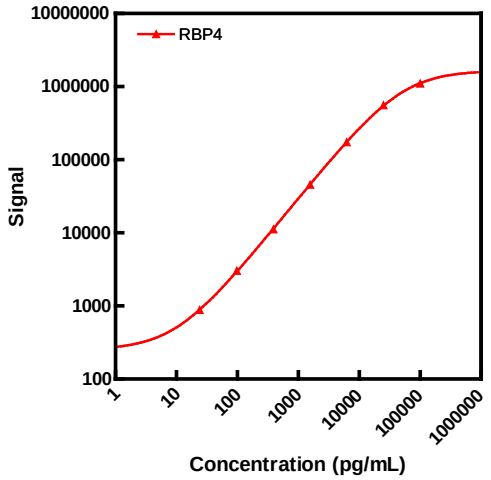
Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

Analysis of Results

MSD DISCOVERY WORKBENCH® software uses least-squares fitting algorithms to generate a standard curve that will be used to calculate the concentration of analyte in the samples. The assays have a wide dynamic range (3–4 logs) which allows accurate quantification without the need for dilution in many cases. The software uses a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting function is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

Typical Data

The following standard curve illustrates the dynamic range of the assay. Actual signals will vary. Best quantification of unknown samples will be achieved by generating a standard curve for each plate using a minimum of 2 replicates of standards.



	RBP4	
Conc. (pg/mL)	Average Signal	%CV
0	170	8.2
24	880	4.3
98	3038	2.5
391	11 163	5.9
1563	45 731	5.7
6250	174 029	5.6
25 000	557 596	6.2
100 000	1 103 480	2.4

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration based on a signal 2.5 standard deviations above the blank (zero calibrator).

	RBP4
Average LLOD (pg/mL)	1.8

Assay Components

Calibrator

The assay calibrator uses recombinant human RBP4 protein expressed in HEK 293 cells.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
RBP4	Mouse Monoclonal	Mouse Monoclonal

References

1. Blaner WS. Retinol-binding protein: the serum transport protein for vitamin A. Endocr Rev 1989 10:308-16.
2. Kirsztajn GM, Nishida SK, Silva MS, Ajzen H, Moura LA, Pereira AB. Urinary retinol-binding protein as a prognostic marker in glomerulopathies. Nephron 2002 Apr;90(4):424-31.
3. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, Kahn BB. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. 2005 Nature 436:356-62.

Summary Protocol

MSD 96-well Human RBP4 Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human RBP4 assay.

Sample and Reagent Preparation

Bring all reagents to room temperature, and thaw the calibrator on ice.

Prepare Blocker A solution.

Prepare 8 standard solutions using the supplied calibrator as described in the "Prepare Standards" section.

Dilute samples 50-fold in Diluent 37 before adding to the plate.

Prepare detection antibody solution by diluting 50X detection antibody 50-fold in Diluent 37.

Prepare 2X Read Buffer T by diluting 4X Read Buffer T 2-fold with deionized water.

Step 1: Add Blocker A Solution

Add 150 µL/well of Blocker A solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 30 minutes.

Step 2: Wash and Add Sample or Calibrator

Wash plate 3 times with 300 µL/well of PBS-T.

Add 50 µL/well of calibrator or diluted sample.

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

Step 3: Wash and Add Detection Antibody Solution

Wash plate 3 times with 300 µL/well of PBS-T.

Add 25 µL/well of 1X detection antibody solution.

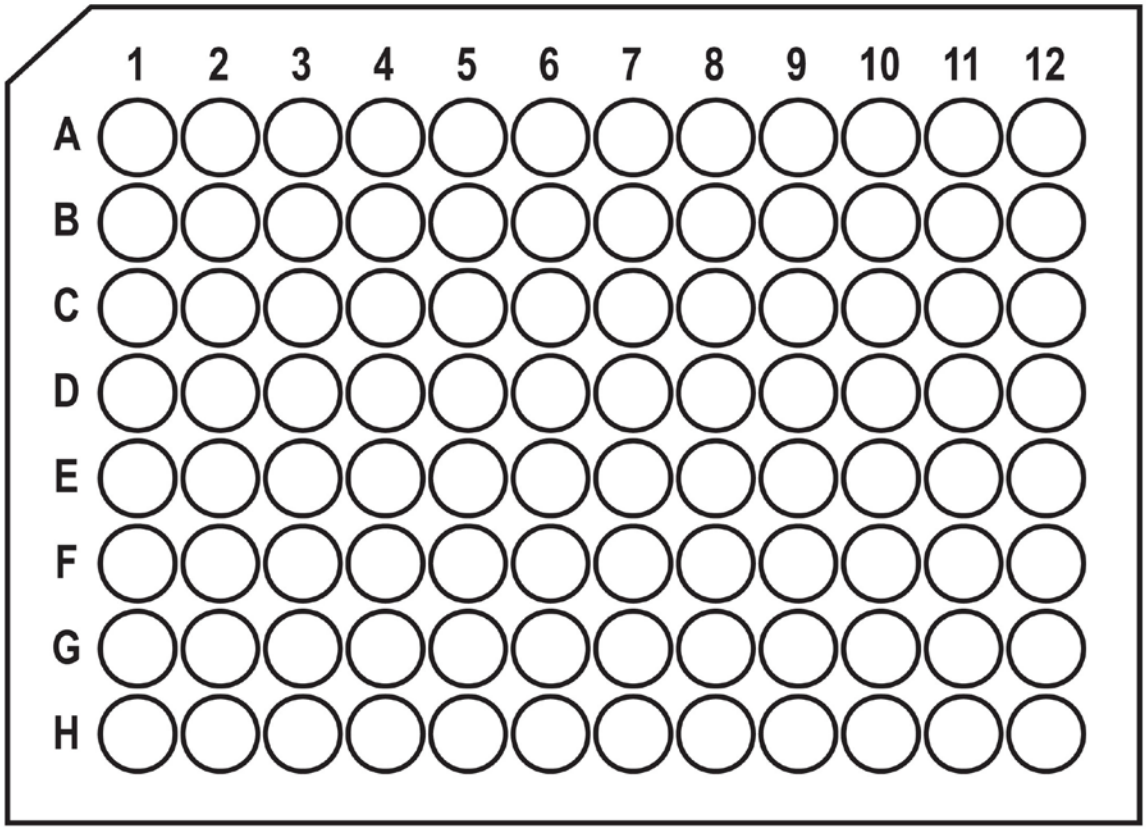
Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 4: Wash and Read Plate

Wash plate 3 times with 300 µL/well of PBS-T.

Add 150 µL/well of 2X Read Buffer T.

Analyze plate on SECTOR Imager.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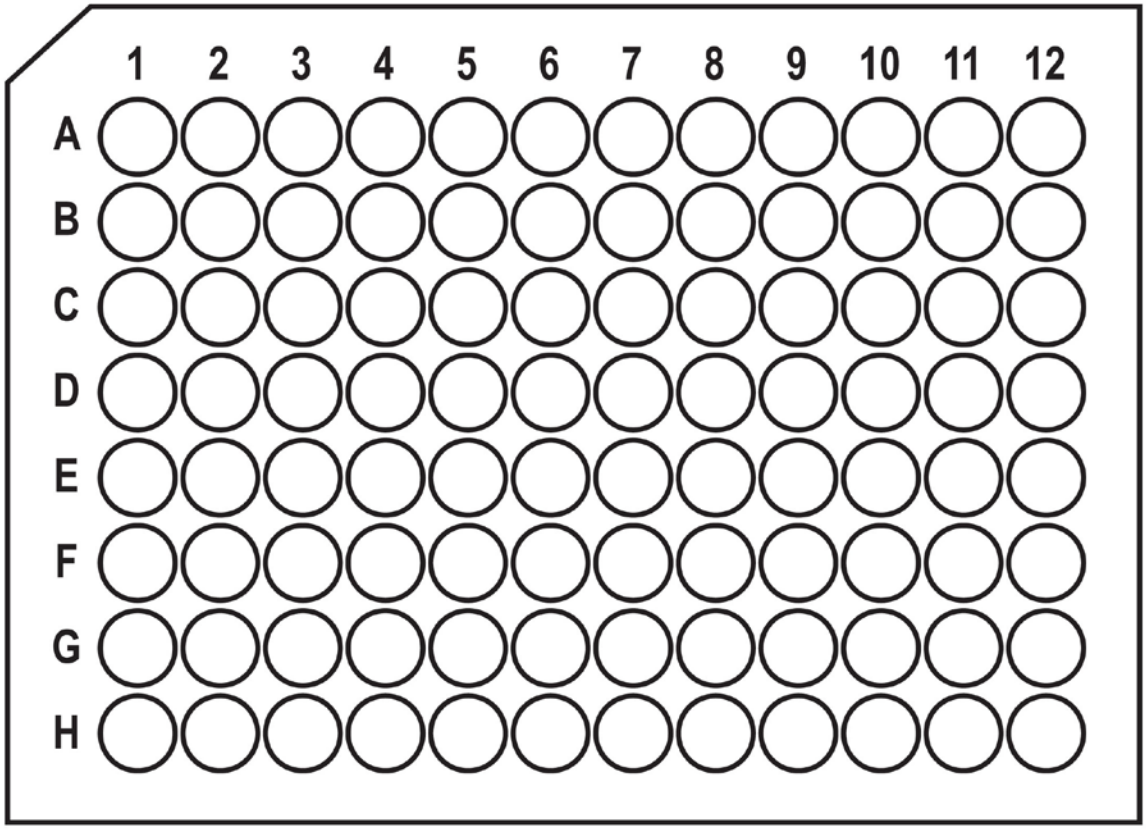
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