# Mouse RBP4 ELISA Kit

**Catalog No**: IRAPKT4023 **Lot No**: SAMPLE

## INTENDED USE

The Mouse RBP4 ELISA kit is designed for detection of mouse RBP4 in plasma, serum, urine, and cell culture supernatants.

www.innov-research.com
Ph: 248.896.0145 | Fx: 248.896.0149
Innovative Research, Inc.

## INTRODUCTION

Serum retinol-binding protein (RBP4) is secreted by the liver and adipocytes and is implicated in systemic insulin resistance. RBP4 transports retinol and circulates in the plasma by binding to the larger transthyretin (TTR) homotetramer, forming a protein complex that reduces renal clearance of RBP4. In insulin-resistant ob/ob mice, urinary fractional excretion of RBP4 was reduced, consistent with increased retention; while TTR level is elevated (1). RBP4 is encoded by the *RBP4* gene that maps to chromosome 10q23-q24 linked to increased risk for type 2 diabetes in different populations (2 - 3). Transgenic overexpression of RBP4 or injection of recombinant RBP4 in normal mice causes insulin resistance. Conversely, genetic deletion of RBP4 enhances insulin sensitivity. Increasing serum RBP4 induces hepatic expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase and impairs insulin signaling in muscle tissue (4). Expression of RBP4 is induced in adipose tissue as a consequence of decreased glucose transporter GLUT4 expression. Increased serum RBP4 is associated with insulin resistance, Type II diabetes, and metabolic syndrome such as obesity, glucose intolerance, dyslipidemia, and hypertension (5 - 6). Plasma RBP4 concentration might be a biomarker of nephropathy and cardiovascular disease in type 2 diabetic subjects (7).

## PRINCIPLE OF THE ASSAY

The Mouse RBP4 ELISA kit is designed for detection of mouse RBP4 in plasma, serum, urine, and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay that measures mouse RBP4 in less than 4 hours. A polyclonal antibody specific for mouse RBP4 has been pre-coated onto a microplate. Mouse RBP4 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for mouse RBP4, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

## CAUTION AND WARNING

- **Prepare all reagents as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

## REAGENTS

- **Mouse RBP4 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against mouse RBP4.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- **Mouse RBP4 Standard:** Mouse RBP4 in a buffered protein base (20 ng, lyophilized).
- **Biotinylated Mouse RBP4 Antibody (70x):** A 70-fold concentrated biotinylated polyclonal antibody against mouse RBP4 (105 µl).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate**): A 100-fold concentrate (80 µl).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

## STORAGE CONDITION

- Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date.
- Store SP Conjugate and biotinylated antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8°C.
- Store standard at 2-8°C before reconstituting with diluent and at -20°C after reconstituting with diluent.

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl and multiple channel).
- Deionized or distilled reagent grade water.

## SAMPLE COLLECTION, PREPARATION AND STORAGE

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:8000 into MIX Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes and remove serum. Dilute samples 1:8000 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes and assay. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

## REAGENT PREPARATION

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x)**: If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2-8°C.
- **Mouse RBP4 Standard:** Reconstitute the 20 ng of Mouse RBP4 Standard with 1 ml of MIX Diluent to generate a standard solution of 20 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (20 ng/ml) 1:2 with MIX Diluent to produce 10, 5, 2.5, 1.25, and 6.25 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

| Standard Point | Dilution | [Mouse RBP4] (ng/ml) |
|---|---|---|
| P1 | Standard (20 ng/ml) | 20.00 |
| P2 | 1 part P1 + 1 part MIX Diluent | 10.00 |
| P3 | 1 part P2 + 1 part MIX Diluent | 5.000 |
| P4 | 1 part P3 + 1 part MIX Diluent | 2.500 |
| P5 | 1 part P4 + 1 part MIX Diluent | 1.250 |
| P6 | 1 part P5 + 1 part MIX Diluent | 0.625 |
| P7 | MIX Diluent | 0.000 |

- **Biotinylated Mouse RBP4 Antibody (70x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:70 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

## ASSAY PROCEDURE

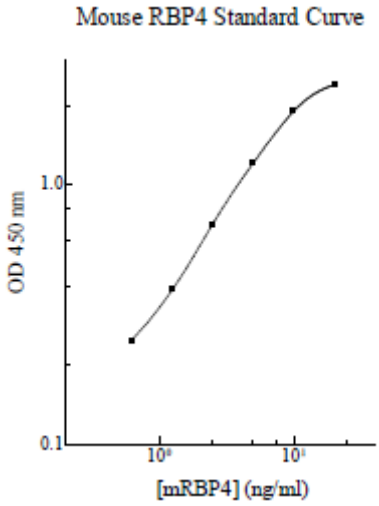
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Mouse RBP4 Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Mouse RBP4 Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 25 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

## DATA ANALYSIS

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

## STANDARD CURVE

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.




## PERFORMANCE CHARACTERISTICS

• The minimum detectable dose of mouse RBP4 is typically ~ 6 ng/ml.
• Intra-assay and inter-assay coefficients of variation were 5.0 % and 7.2 % respectively.

## LINEARITY

| | Average Percentage of Expected Value | |
|---|---|---|
| Sample Dilution | Plasma | Serum |
| 1:2000 | 89% | 88% |
| 1:8000 | 97% | 98% |
| 1:16000 | 104% | 103% |

## RECOVERY

| | |
|---|---|
| Standard Added Value | 1 – 10 ng/ml |
| Recovery % | 83 - 111 % |
| Average Recovery % | 98% |

## CROSS-REACTIVITY

| Species | % Cross Reactivity |
|---|---|
| Beagle | None |
| Bovine | None |
| Monkey | None |
| Mouse | 100% |
| Rat | 5% |
| Swine | None |
| Human | None |
| Rabbit | None |

- 10% FBS in culture media will not affect the assay.

## REFERENCES


(1) Mody N *et al.* (2008) *Am. J. Physiol Endocrinol Metab*. 294(4): E785-793
(2) Meigs JB *et al.* (2002) *Diabetes* 51:833–840
(3) Duggirala R *et al*. (1998) *Diabetes* 47 (Suppl. 1): A170
(4) Yang Q *et al.* (2005) Nature 436 (7049): 356-362
(5) Graham T.E. *et al.* (2006) *N. Engl. J. Med.* 354:2552-2563
(6) McTernan PG *et al.* (2007*) J. Clin. Endocrinol. Metab*. 92:2430 –2432
(7) Cabre A *et al.* (2007) J. Intern Med. 262(4): 496-503

# Assay Template

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| 12 | | | | | | | | |
| 11 | | | | | | | | |
| 10 | | | | | | | | |
| 9 | | | | | | | | |
| 8 | | | | | | | | |
| 7 | | | | | | | | |
| 6 | | | | | | | | |
| 5 | | | | | | | | |
| 4 | | | | | | | | |
| 3 | | | | | | | | |
| 2 | | | | | | | | |
| 1 | | | | | | | | |

*This material is intended for research use or for further manufacture of in-vitro diagnostic products only. The material is not for use in products licensed under Sec. 351 of the PHS act. The user agrees to indemnify and hold Innovative Research, Inc. harmless for any use of this product other than that specifically stated.*

www.innov-research.com
Ph: 248.896.0145 | Fx: 248.896.0149

**Innovative Research, Inc.**