MOUSE/RAT RETINOL BINDING PROTEIN 4 (RBP-4) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE OR RAT RBP-4 CONCENTRATIONS IN SERUM AND PLASMA

PURCHASE INFORMATION:

ELISA NAME	MOUSE/RAT RBP-4 ELISA
Catalog No.	SK00107-05
Lot No.	
Formulation	96T
Standard range	0.32 - 1000 ng/ml
Dynamic Range	1.6 – 200 ng/ml
Sensitivity	1.6 – 2 ng/ml
Sample Volume	50 µl
Dilution Factor	50 **(Optimal dilutions should be determined by each laboratory for each application)**
Sample Type	Serum and Plasma
Specificity	Mouse and Rat RBP-4
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 - 8° C

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

Mouse RBP-4 ELISA employs the quantitatively competitive enzyme immunoassay technique in which RBP-4 present in samples compete with a fixed amount of biotinylated mouse RBP-4 for sites on purified rabbit IgG specific against mouse RBP-4. During the incubation, the rabbit IgG becomes bound to the goat anti-rabbit IgG pre-coated onto the microplate. Following a wash to remove any unbound antibody, standard, samples and biotin conjugate, a Streptavidin conjugated to horseradish-peroxidase (HRP) is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in inverse proportion to the amount of mouse RBP-4 bound in the initial step. The sample values are then read off the standard curve.

Mouse RBP-4 ELISA has been shown to accurately quantify the recombinant full length and natural mouse RBP-4. Results obtained using natural mouse RBP-4 showed dose response curves that were parallel to the standard curves obtained using the kit standards.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute reagents with those from other lots or sources.
_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
R-Microplate – 96 well microplate pre-coated with polyclonal anti-rabbit IgG.	**RM01**	**1 plate**
RBP-4 Standard – 1000 ng/vial of recombinant mouse RBP-4 in a buffered protein base with preservative; lyophilized.	**107-05-01**	**1 vial**
Antibody Concentrate – 350 µl/vial, 10-fold concentrate of polyclonal purified IgG against mouse RBP-4 with preservative; lyophilized.	**107-05-02**	**1 vial**
Biotin Concentrate – 350 µl/vial, 10-fold concentrate of biotinylated mouse RBP-4 with preservative; lyophilized.	**107-05-03**	**1 vial**
Positive Control – one vial of recombinant mouse RBP-4; lyophilized (optional).	**107-05-04**	**1 vial**
Streptavidin-HRP Conjugate – 240 µL/vial, 50-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB06**	**1 bottle**
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution – 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store kit at 2 – 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control, Antibody Concentrate and Biotin Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard (stock), Biotin concentrated solution and Antibody concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. Reconstituted Biotin Solution (350 µl) CAN NOT BE STORED at 2 – 8° C. Streptavidin-HRP Conjugate 50-fold concentrated solution (protect from light) and other components may be stored at 2 – 8° C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples may need a 50-fold dilution. A suggested 10-fold dilution is 10 µL sample + 90 µL Dilution Buffer. To make 50-fold dilution: 50 µL of 10-fold diluted sample + 200 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.**

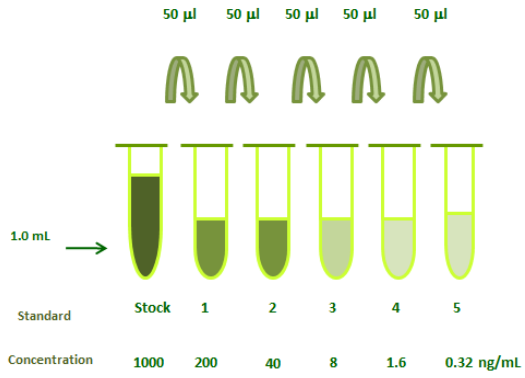
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

RBP-4 Standard - Reconstitute the RBP-4 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 1000 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 200 µL of Dilution Buffer into tubes #1 to #5. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **1000 ng/mL** standard serves as the high standard.

Tube	Standard	Dilution Buffer	Concentration
stock	powder	1.0 ml	1000 ng/ml
# 1	50µl of stock	200µl	200 ng/ml
# 2	50µl of 1	200µl	40 ng/ml
# 3	50µl of 2	200µl	8 ng/ml
# 4	50µl of 3	200µl	1.6 ng/ml
# 5	50µl of 4	200µl	0.32 ng/ml



Positive Control – Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. *Note: Positive Control should be used immediately. It can be reused within a few days if stored at -20° C or -70° C.*

Antibody Concentrate - Reconstitute the Antibody Concentrate with 350 µl of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer it to 3.15 mL of Dilution Buffer to prepare 1x Antibody Solution.

Biotin Concentrate – Reconstitute the Biotin Concentrate with 350 µl of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer it to 3.15 mL of Dilution Buffer to prepare 1x Biotin Solution.

Streptavidin-HRP Conjugate – Transfer 240 µl of 50-fold concentrated stock solution to 11.76 mL of **HRP Diluent Solution (DB06)** to prepare working solution. *Note: 1x working solution of Streptavidin-HRP Conjugate should be used within a few days (protect from light).*

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. ***FOR BLANK WELLS DO NOT ADD ANY SOLUTIONS EXCEPT HRP. DO NOT ADD ANTIBODY OR BIOTINYLATED SOLUTION INTO BLANK WELLS.***
4. Add 50 µl per well of Dilution Buffer to Total Binding wells. Add 50 µl per well of Standard solutions from #5 to S in reverse order of serial dilution. Add 50 µl per well of Positive Control solution. Add 50 µl per well of samples to unused wells. *Note: Samples, Standards, Blanks and Positive Control should be assayed in duplicate.*
5. Add 25 µl per well of **1x Antibody Solution** into total binding, standard, positive control and sample wells. Cover with plate sealer and incubate on microplate shaker (250-300rpm) at room temperature for 2 hours. ***Note: DO NOT ASPIRATE AND WASH PLATE. PROCEED IMMEDIATELY TO THE NEXT STEP.***
6. Add 25 µl per well of **1x Biotin Solution** into total binding, standard, positive control and sample wells. Cover with plate sealer and incubate on microplate shaker (250-300rpm) at room temperature for 2 hours. ***Note: DO NOT ADD Biotin Solution to Blank wells.***
7. Aspirate wells and wash 4 times with 300 µl of **1x Wash Buffer**. Blot plate on absorbent paper to remove any residual buffer.
8. Add 100 µl of **Streptavidin-HRP Conjugate working solution** to each well. Incubate on microplate shaker (250-300rpm) at room temperature for one hour. **Protect from light.**
10. Aspirate and wash as step 7.
11. Add 100 µl of **Substrate Solution** to each well. Incubate on microplate shaker (250-300rpm) at room temperature for 5-15 minutes. **Protect from light**.
12. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
13. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard solution, positive control and samples, and subtract the average blank optical density. It is recommended

to use software capable of generating a four parameter logistic (4-PL) curve-fit. The standard curve shows relationship between standard concentrations and corresponding O.D absorbance. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Mouse RBP-4 Standard (ng/mL)	Average OD450 (Corrected)
Blank	0 (0.086)
Total Binding	0.845
0.32	0.811
1.6	0.699
8	0.505
40	0.211
200	0.095
1000	0.044

- Lot No.:
- Positive Control:

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant mouse RBP-4.

SENSITIVITY

The minimum detectable dose (MDD) of RBP-4 was 1.6 – 2 ng/mL.

REFERENCES

1: Bobbert P, et al. Increased plasma retinol binding protein 4 levels in patients with inflammatory cardiomyopathy. Eur J Heart Fail. 2009 Dec;11 (12):1163-8.

2: Ingelsson E, et al. Circulating retinol-binding protein 4, cardiovascular risk factors and prevalent cardiovascular disease in elderly. Atherosclerosis. 2009 Sep; 206(1):239-44. Epub 2009 Mar 11.

3: Laudes M,et al. Human fetal adiponectin and retinol-binding protein (RBP)-4 levels in relation to birth weight and maternal obesity. Exp Clin Endocrinol Diabetes. 2009 Mar;117(3):146-9. Epub 2008 Dec 3.

SPECIFICITY

Mouse RBP-4 ELISA kit recognizes recombinant and endogenous mouse RBP-4. Data also indicates that rat serum samples competitively bind to the antibody that was used in this kit formulation condition. Its linear dilution curves were parallel to the standard curves obtained using the ELISA standard. Thus, rat serum samples cross-react with mouse RBP-4 ELISA kit.

Proteins	Cross-reactivity
Mouse RBP-4, full length	100%
Human RBP-4, full length (active form)	70%
Human RBP-4 isolated from urine (non-active form)	5%
Mouse Letin	0
Mouse Adiponectin	0
Mouse FABP-4	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⇓
Add 50 µl of standard dilutions, samples, or positive control to the well. Add 25 µl of 1x Antibody Solution to each well. Incubate 2 hours on the plate shaker at RT. ***DO NOT ASPIRATE OR WASH PLATE. PROCEED IMMEDIATELY TO NEXT STEP.***
⇓
Add 25 µl 1x Biotin Solution to each well. Incubate 2 hours on the plate shaker at RT.
⇓
Aspirate and wash 4 times.
⇓
Add 100 µl Streptavidin-HRP conjugate working solution to all wells. Incubate one hour on the plate shaker at RT. **Protect from light.**
⇓
Aspirate and wash 4 times.
⇓
Add 100 µl Substrate Solution to each well. Incubate 5-15 min on the plate shaker at RT. **Protect from light.**
⇓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.