HUMAN RETINOL BINDING PROTEIN-4 (RBP-4) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN RBP-4 CONCENTRATIONS IN SERUM AND PLASMA.

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

PURCHASE INFORMATION:

ELISA NAME	HUMAN RBP-4 ELISA
Catalog No.	SK00107-06
Lot No.:	
Formulation	96 T
Standard range	49-50000 pg/ml
Sensitivity	25 pg/ml
Sample Volume	100 µl of diluted samples
Dilution Factor	*2000~4000 (Optimal dilutions should be determined by each laboratory for each application)*
Sample Type	Serum, EDTA plasma
Specificity	Human RBP-4
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	4 °C

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INTRODUCTION

Human RBP-4 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human RBP-4 in serum and EDTA plasma. It contains recombinant human RBP-4 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human RBP-4. Results obtained with naturally occurring RBP-4 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human RBP-4.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human RBP-4 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any RBP-4 present is bound by the immobilized antibody. After washing away any unbound substances, an antibody specific for RBP-4 is added to the wells. Following a wash to remove any unbound antibody reagent, ARIGHRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of RBP-4 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ 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.

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.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
RBP-4-Microplate – 96 well microplate precoated with an anti-human RBP-4 antibody	**107-06-01**	**1 plate**
RBP-4 Standard – 50 ng/vial of recombinant Human RBP-4 in a buffered protein base with preservatives; lyophilized.	**107-06-02**	**1 vial**
RBP-4 Antibody Concentrate – 120µl / vial, 100-fold concentrated of antibody against Human RBP-4 with preservatives; lyophilized.	**107-06-03**	**1 vial**
Positive Control – one vial of recombinant Human RBP-4 , lyophilized (optional)	**107-06-04**	**1 vial**
Anti Rabbit IgG HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of ARIG conjugate to HRP	**ARIGHRP**	**1 vial**
Dilution Buffer - 60 mL of buffered protein based solution with preservatives	**DB08**	**2 bottles**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution- 11ml / vial of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution - 11 ml /vial of 0.5M HCl	**S-STOP**	**1 bottle**

Plate Sealer	**EAPS**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8°C for up to 6 months. For longer storage, unopened Standard, Positive Control, Detection Antibody Concentrate should be stored at -20°C or -70°C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard and Antibody Solution SHOULD BE STORED at -20°C or -70°C for up to one month. ARIG-HRP Conjugate 100-fold concentrate and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. 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, with the correction wavelength set at 540 nm or 570 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.

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.
Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

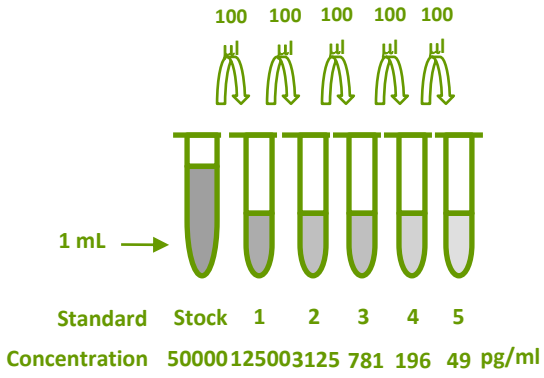
Serum and EDTA plasma samples may require a 2000~4000 fold dilution. A suggested 2000-fold dilution is 25 µL sample + 975 µL Dilution Buffer. Following 20 µl of 40-fold diluted samples + 980 µl Dilution Buffer. A suggested 4000-fold dilution is 12.5 µL sample + 987.5 µL Dilution Buffer. Following 20 µl of 80-fold diluted samples + 980 µl of Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application.**
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 Wash Buffer.

RBP-4 Standard - Refer to vial label for reconstitution volume. Reconstitute the **RBP-4** Standard with 1.0 ml of Dilution Buffer. This reconstitution produces a stock solution of 50000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 300 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 50000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

STANDARD TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	50000 pg/ml
# 1	100µl of stock	300µl	12500 pg/ml
# 2	100µl of 1	300µl	3125 pg/ml
# 3	100µl of 2	300µl	781 pg/ml
# 4	100µl of 3	300µl	196 pg/ml
# 5	100µl of 4	300µl	49 pg/ml



RBP-4 Antibody Concentrate - Reconstitute the Antibody Concentrate with 120 µl of Dilution Buffer to produce a 100-fold concentrated stock solution. Transfer it to 11.88 mL of Dilution Buffer to prepare 1x Antibody working solution.

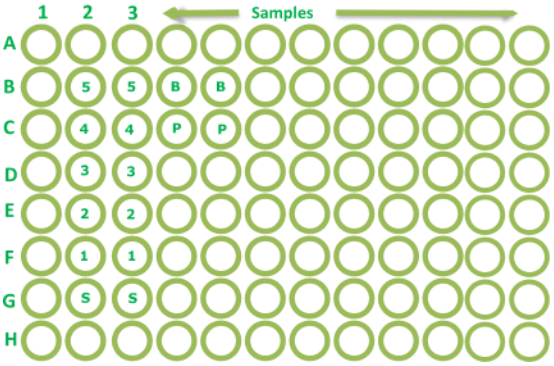
Anti Rabbit IgG-HRP Conjugate - Transfer 120 µl of 100-fold concentrated stock solution to 11.88 ml of Dilution Buffer to prepare working solution. **Note:** 1x working solution of Anti Rabbit IgG HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used immediately.

ASSAY PROCEDURE

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

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch containing the desiccant pack, reseal.
3. Leave well B4 and B5 as Blank. Add 100 µl per well of Dilution Buffer.
4. Add 100 µl per well of standard solution from #5 to #S (reverse order of serial dilution) to the appropriate wells (B2, B3 to G2, G3). Add 100 µl per well of Positive control into well C4 and C5. Add 100 µl per well of samples into appropriate wells. Cover or seal the plate and incubate at room temperature for 2 hours on microplate shaker (250 rpm). Note: Standard, Blank and PC should be assayed in duplicates.
5. Aspirate wells and wash 4 times with 300 µl of 1x Assay Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
6. Add 100 µl per well of 1x Antibody working solution. Cover or seal the plate and incubate at room temperature for 2 hours on microplate shaker (250 rpm).
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Anti Rabbit IgG-HRP Conjugate** working solution. Cover or seal the plate and incubate at room temperature for 1 hour on microplate shaker. **Protect from light**.
11. Repeat the aspiration/wash as in step 5.
12. Add 100 µL of Substrate Solution to each well. Incubate for 3-7 minutes at room temperature. **Protect from light**.
13. 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.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, PC , 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.

CALIBRATION

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

SENSITIVITY

The minimum detectable dose (MDD) of human RBP-4 was 25 pg/mL.

TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.085)
49	0.127
196	0.349
781	0.791
3125	1.291
12500	1.745
50000	1.969

Lot No.: 20110419
Positive control: 1891 ~3512 pg/mL

SPECIFICITY

This assay recognizes both natural and recombinant human RBP-4. The factors listed below were prepared at 2500 ng/mL in Dilution Buffer, and assayed for cross reactivity. No significant cross-reactivity or interference was observed.

PROTEINS	CROSS-REACTIVITY
Human RBP-4	100
Human sRAGE	0
Human Visfatin	0
Human FABP-4	0
Human Adiponectin	0
Human FTO	0
Human Vaspin	0

RESEARCH DATA

SAMPLES	RBP-4 (µG/ML)
Normal Subjects (n=7)	25.94 ± 5.30
CVD (n=8)	41.06 ± 3.81

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl 1x Antibody Working Solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Anti Rabbit IgG HRP conjugate working solution to all wells. Incubate 1 hour on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 3-7 min on the bench top. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min