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

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

PRODUCT INFORMATION:
THIS IS FOR ONE TIME USE ONLY.

ELISA NAME	HUMAN RBP-4 ELISA KIT
Catalog No.	SK00107-07
Lot No.:	
Formulation	96 T
Standard range	156 – 40000 pg/mL
Sensitivity	50 pg/mL
Sample Volume	100 µL of diluted samples
Dilution Factor	*4000 ~ 8,000 (Optimal dilutions should be determined by each laboratory for each application)*
Sample Type	Serum, EDTA Plasma
Specificity	Human RBP-4
Calibration	Human RBP-4 Rec. (HEK293)
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 - 8° C for 1 month. See page 2-3 for more detailed
This kit contains sufficient materials to run 35~ 40 samples duplicated provided that assay is run according to protocol.	

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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DESCRIPTION

This Human RBP-4 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human RBP-4 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human RBP-4 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural RBP-4 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human RBP4. The capture antibody can bind to the human bioactive RBP4 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human RBP4 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human RBP4 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix 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.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
RBP-4-Microplate – 96 well microplate precoated with an anti-human RBP-4 monoclonal antibody.	107-07-01	1 plate
RBP-4 Standard – refer to lot of recombinant human RBP-4 in a buffered protein base with preservative; lyophilized.	107-07-02	1 vial
Detection Antibody Concentrate – 1.2 mL/vial of 10-fold concentrated biotinylated antibody against RBP4 lyophilized.	107-07-03	1 vial
Positive Control – one vial of recombinant human RBP-4; lyophilized (optional).	107-07-04	1 vial
Streptavidin-HRP Conjugate – 120 µL of 100-fold concentrated solution of Streptavidin conjugate to HRP.	SAHRP	1 vial
Dilution Buffer Concentrate - 45 mL of buffered protein based solution with preservative.	DB01	2 bottles
Antibody Diluent Solution - 12 mL of buffered protein based solution with preservative.	DB02	1 bottle
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	DB08B	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 0.25M HCl.	S-STOP	1 bottle
Plate Sealer	EAPS	1 piece
Plastic Pouch	P01	1 piece

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 1 month. longer storage up to 10 months, unopened Standard, Positive Control, Detection Antibody, Dilution Buffer

and Antibody & HRP Diluent Solution should be stored at -20° C.
Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2 ~ 8°C.
Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

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 EDTA plasma samples may require a 4000 ~ 8000-fold dilution. A suggested 100-fold dilution is 5 µL sample + 495 µL 1x Dilution Buffer. Then, to make a 4000-fold dilution is 6 µL of 100-fold diluted samples + 234 µl 1x Dilution Buffer.
To make a 8000-fold dilution is 4 µL of 100-fold diluted sample + 316 µL of 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 refer to lot of Dilution Buffer. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer. The **40000 pg/mL** standard serves as the high standard. The 1x Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	Refer to lot	XXXX
# 1	Refer to lot	Refer to lot	40000 pg/ml
# 2	100µl of 1	300µl	10000 pg/ml
# 3	100µl of 2	300µl	2500 pg/ml
# 4	100µl of 3	300µl	625 pg/ml
# 5	100µl of 4	300µl	156 pg/ml

Positive Control - Reconstitute the Positive Control with refer to lot of Dilution Buffer.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.2 mL of **Antibody Diluent Solution (DB02)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Antibody Diluent Solution (DB02)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent Solution (DB08B)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold

concentrated stock solution to prepare working solution **(protect from light).**

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL per well of Dilution Buffer to Blank wells.
3. Add 100 µL of standard dilutions, positive control, or samples per well. Cover with the plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of Detection Antibody working solution to each well. Cover with the plate sealer. Incubate for 90 minutes on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 45 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for refer to lot on microplate shaker at room temperature. **Protect from light.**
10. 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.
11. Determine the optical density of each well using a microplate reader set to 450 nm within 2 min.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log or 4-parameter curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

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

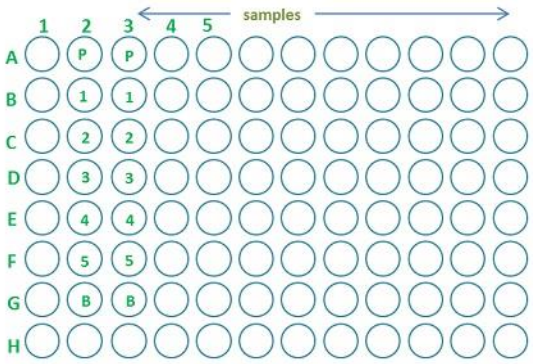
STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.112)
156	0.066
625	0.273
2500	1.024
10000	2.418
40000	2.959

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human RBP4 (HEK293)	100
Human RBP4 (NS0)	100
Mouse RBP4 (HEK293)	0
Human Uromodulin	0
Human sRAGE	0
Human Visfatin	0
Human FABP-4	0
Human Adiponectin	0

Use 5 µL of human serum or plasma samples to prepare 1: 4K or 1:8K dilution.

		Final Dilution
5 µL of human sample	495 µL of 1x Dilution Buffer (DB01)	**100**
6 µL of 100-fold diluted sample solution	234 µL of 1x Dilution Buffer (DB01)	**4000 (4K)**
5 µL of 100 - fold diluted sample solution	395 µL of 1x Dilution Buffer (DB01)	**8000 (8K)**



SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate 3 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 90 minutes on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin HRP conjugate working solution to each well. Incubate 45 min on the plate shaker at RT. **Protect from light**.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate refer to lot on the plate shaker at RT. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read at 450 nm within 2 min.