

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

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



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

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

PRODUCT INFORMATION:

THIS IS FOR ONE TIME USE ONLY.

ELISA NAME	HUMAN RBP-4 ELISA KIT
Catalog No.	SK00107-08
Lot No.:	
Formulation	96 T
Standard range	312.5 – 40000 pg/mL
Sensitivity	50 pg/mL
Sample Volume	100 µL of diluted samples
Dilution Factor	4000 ~ 8,000 (<i>Optimal dilutions should be determined by each laboratory for each application</i>)
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.	

ORDER CONTACT:

AVISCIERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA

Tel: (408) 982 0300

Fax: (408) 982 0301

Email: Sales@AvisceraBioscience.com

Info@AvisceraBioscience.com

www.AvisceraBioscience.com

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 monoclonal 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 an antibody specific for human RBP-4. The capture antibody can bind to the human RBP-4 in the standard and samples. After washing the plate of any unbound substances, an antibody-HRP conjugate against human RBP-4 is added to the wells. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human RBP-4 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.	107-08-01	1 plate
RBP-4 Standard – refer to lot of recombinant human RBP-4 in a buffered protein base with preservative; lyophilized.	107-08-02	1 vial
Detection Antibody-HRP Conjugate – 105 µL/vial of 100-fold concentrated solution of antibody conjugated to HRP against RBP4.	107-08-03	1 vial
Positive Control – one vial of recombinant human RBP-4; lyophilized (optional).	107-08-04	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
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, Dilution Buffer and Antibody Diluent Solution should be stored at -20° C. Antibody-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 $\leq -20^{\circ}$ 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 $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Optimal: 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 μ L sample + 495 μ L 1x Dilution Buffer. Then, to make a 4000-fold dilution is 6 μ L of 100-fold diluted samples + 234 μ L 1x Dilution Buffer.

To make a 8000-fold dilution is 4 μ L of 100-fold diluted sample + 316 μ L 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 4-fold 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	100 μ l of 1	300 μ l	10000 pg/ml
# 3	100 μ l of 2	300 μ l	2500 pg/ml
# 4	100 μ l of 3	300 μ l	625 pg/ml

Positive Control - Reconstitute the Positive Control with refer to lot of Dilution Buffer. **Note:** Positive Control could be reused within a few days if stored at -20° C or -70° C.

Detection Antibody-HRP Conjugate - Pipette 10.395 mL of Antibody Diluent Solution into a 15 mL centrifuge tube and transfer 105 μ L of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Detection Antibody-HRP conjugate should be used within a few hours (**protect from light**). **DO NOT FREEZE.**

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. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 μ l per well of 1x Dilution Buffer to

Blank wells.

4. Add 100 µl per well of standard dilutions from #8 to #1 (reverse order of serial dilution), positive control and samples. Cover with plate sealer and incubate at room temperature for 2 hours on microplate shaker (350 rpm).
5. Aspirate wells and wash 4 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
6. Add 100 µl per well of 1x Detection Antibody-HRP conjugate working solution. Cover with plate sealer and incubate at room temperature for 1 hour on microplate shaker (250 rpm).
Protect from light.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 10-15 minutes on microplate shaker at room temperature. **Protect from light.**
9. 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 2 minutes using a microplate reader set to 450 nm.

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.

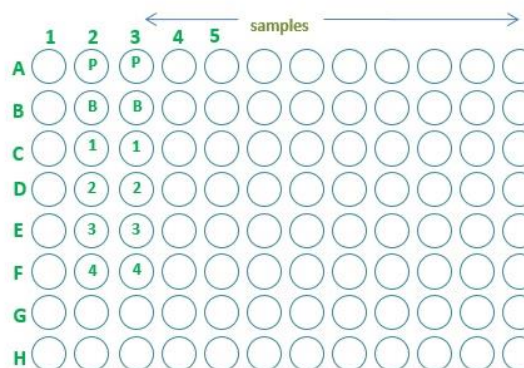
TYPICAL DATA

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 (0.077)
625	0.079
2500	0.365
10000	1.599
40000	2.499

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)

Use a multi-channel Pipette to perform sample dilution on a 96 wells PCR Plate or Low Binding 96 wells ELISA plate

		Final Dilution
10 µL of human sample	Add 190 µL of 1x Dilution Buffer (DB01) by multi-channel pipette to PCR plate	20
Transfer 10 µL of 20-fold diluted sample solution by multi-channel pipette on PCR Plate	Add 190 µL of 1x Dilution Buffer (DB01) by a Multi-Channel Pipette on PCR Plate	400
Transfer 10 µL of 400 -fold diluted sample solution by a multi-channel pipette to each sample assay well on RBP4 Microplate 107-08-01	Add 90 µL of 1x Dilution Buffer (DB01) by a Multi-Channel Pipette to each sample assay well on RBP4 Microplate 107-08-01	4000 (4K)

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl per well 1x Detection Antibody-HRP working solution to each well. 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 10-15 min on the plate shaker at RT. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 2 min.

Human Retinol Binding Protein 4 ELISA Kit SK00170-08
 Standard Range: 0.625 ~ 40 ng/mL
 Sensitivity: 50 pg/mL Specificity: Human
 Calibration: rh RBP4 (HEK293)

