Human Retinol-binding Protein 4 ELISA

Cat. No.: BA1004

Enzyme Immunoassay for the quantitative determination of Retinol-binding Protein 4 (RBP4) in human serum and plasma.

Retinol-binding Protein 4 (RBP4) is a member of the lipocalin family and the major transport protein of the hydrophobic molecule retinol, also known as vitamin A, in the circulation.[1] Retinol-binding Protein 4 (RBP4) is elevated in serum and adipose tissue (AT) in obesity-induced insulin resistance and correlates inversely with insulin-stimulated glucose disposal. Serum RBP4 levels correlated inversely with glucose disposal and insulin-mediated suppression of lipolysis, FFA, and EGP.[2]

High levels of serum RBP4 are associated with chronic kidney disease (CKD) and urine BRP4 is the most sensitive biomarker of proximal renal tubule function.

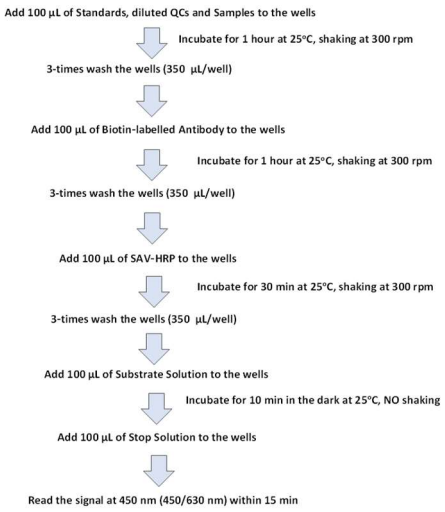
PRINCIPLE OF RBP4 ELISA

The microtiter plate is coated with the antibody specifically binding the Retinol-binding Protein 4. The human serum or plasma is incubated in the plate with the capture antibody.

The specimen is washed out and the specifically bound protein is incubated with biotin-labelled detection antibody. Following another washing step, Streptavidin-HRP conjugate is added into the well.

Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H_2SO_4).

The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of RBP4 in the specimen. The concentration of RBP4 in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.



Kit Contents

Item	*Qty.*
Antibody Coated Microtiter Plate	96 wells
Biotin-labelled Antibody	13 mL
Streptavidin-HRP Conjugate	13 mL
Master Standard (lyophilized)	1 vial
Quality Control A (human serum, lyophilized)	1 vial
Quality Control B (human serum, lyophilized)	1 vial
Dilution Buffer	13 mL
Wash Buffer 15x conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

MATERIAL REQUIRED BUT NOT SUPPLIED

1. Glassware and test tubes
2. Microtiter plate washer
3. Precision pipettes (various volumes) with tips
4. Orbital shaker
5. Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation

WARNINGS AND PRECAUTIONS

1. For research use only
2. For professional laboratory use
3. The reagents with different lot numbers should not be mixed
4. To prevent cross sample contamination, use disposable labware and pipette tips
5. To protect laboratory stuff, wear protective gloves and protective clothing
6. The substrate solution should remain colourless, keep it protected from light
7. The test should be performed at standard laboratory conditions (temperature 25°C ± 2°C).

STORAGE CONDITIONS

1. The kit must be stored at 2 – 8°C.
2. The opened components can be stored for one week at 2 – 8°C.

PREPARATION OF REAGENTS

- Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination.
- All reagents and samples should be allowed to reach the temperature 25°C ± 2°C.

Preparation of Standards

Reconstitute lyophilized Human RBP4 Master Standard in Dilution Buffer, for the volume information see the Certificate of Analysis. Let it rehydrate for 15 min. The concentration of human RBP4 in Master Standard is 120 ng/mL.

Prepare set of Standard solution as follows:

Use the Master Standard to produce a dilution series (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 100 ng/mL (lyophilised)	1000 µL	120 ng/mL
Std2	300 µL of Std1	300 µL	60 ng/mL
Std3	300 µL of Std2	300 µL	30 ng/mL
Std4	300 µL of Std3	300 µL	15 ng/mL
Std5	300 µL of Std4	300 µL	7.5 ng/mL
Std6	300 µL of Std5	300 µL	3.75 ng/mL
Blank	-	300 µL	0 ng/mL

Preparation of Quality Control A and B

Reconstitute the lyophilized human serum Quality Controls in deionized/distilled, for the volume information see the Certificate of Analysis. Let the QCs rehydrate for 15 min and dilute them 1:750 in Dilution Buffer, prior to use, see Preparation of samples (use the two-step dilution).

Preparation of Wash Buffer 1x

Prepare a working solution of Wash Buffer by adding 50 mL of Wash Buffer 15x conc. to 700 mL of deionized/ distilled water (dH_2O). Mix well. Store at 4°C for two weeks or at -20°C for long term storage.

Preparation of samples

Human serum or plasma may be used with this assay. For long-term storage the samples should be frozen at minimum -70°C. Lipemic or haemolytic samples may cause false results.

Recommended dilution of samples is 1:750. It is recommended to use the two-step dilution.
Dilution A (25x) for both singlets and duplicates: 5 µL of samples + 120 µL of Dilution Buffer.
Dilution B (30x): 5 µL of Dilution A + 145 µL of Dilution Buffer, for singlets; 9 µL of Dilution A + 261 µL of Dilution Buffer for duplicates.

Do not store the diluted samples.

ASSAY PROCEDURE

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µL of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for **1 hour** at 25°C ± 2°C, shaking at 300 rpm.
3. Wash the wells 3-times with 1x Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µL of Biotin-labelled Antibody into each well. Incubate for **1 hour** at 25°C ± 2°C, shaking at 300 rpm.
5. Wash the wells as described in point 3.
6. Pipette 100 µL of Streptavidin-HRP into each well. Incubate for **30 min** at 25°C ± 2°C, shaking at 300 rpm.
7. Wash the wells as described in point 3.
8. Pipette 100 µL Substrate solution, incubate for **10 min,** at 25°C ± 2°C. Avoid exposure to the light during this step.
9. Pipette 100 µL of STOP solution.
10. Read the signal at 450/630 nm within 15 min.

PERFORMANCE CHARACTERISTICS

Samples used in the tests were diluted 1:750 as recommended and assayed. The results are multiplied by dilution factor.

1. Sensitivity

The limit of detection, defined as a concentration of human RBP4 giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 0.94 ng/mL of sample.

2. Precision

Intra-assay

Sample	Mean (µg/mL)	SD	CV (%)
1	24.5	2.8	12
2	22.1	2.6	12

Inter-assay (Run – to – run)

Sample	Mean (µg/mL)	SD	CV (%)
1	40.5	4.2	10
2	35.7	1.8	5

3. Accuracy

Dilution linearity

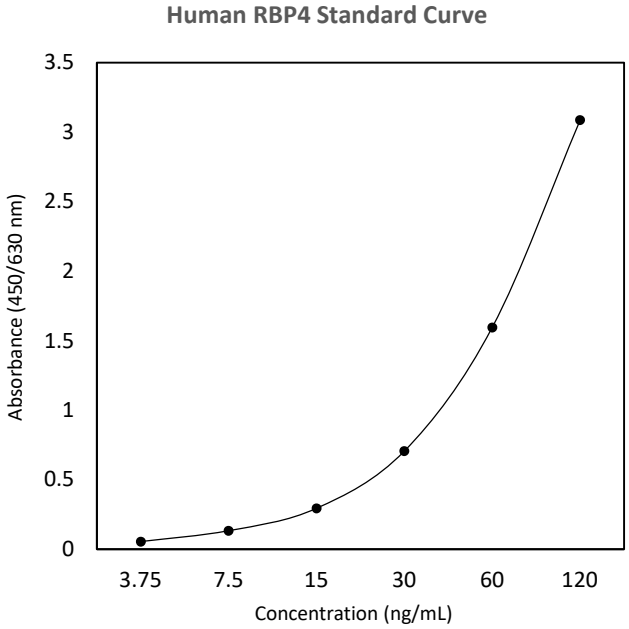
Sample	Dilution	Measured concentration (µg/mL)	Expected concentration (µg/mL)	Yield (%)
1		22.4	-	-
	2x	9.1	11.2	81
	4x	4.8	5.6	85
	8x	3.0	2.8	108
2		23.0	-	-
	2x	9.7	11.5	84
	4x	5.1	5.8	88
	8x	3.2	2.9	110

Spiking Recovery

Sample	Spike (µg /mL)	Measured concentration (µg/mL)	Expected concentration (µg/mL)	Yield (%)
1	-	19.1	-	-
	+ 9.4	25.4	28.5	89
	+ 4.7	22.1	23.8	93
	+2.3	24.4	21.4	114

Typical standard curve

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.



RESOURCES

[1] Steinhoff JS, Lass A, Schupp M. Biological Functions of RBP4 and Its Relevance for Human Diseases. Front Physiol. 2021 Mar 11;12:659977. doi: 10.3389/fphys.2021.659977. PMID: 33790810; PMCID: PMC8006376.

[2] Kilicarslan M, de Weijer BA, Simonyté Sjödin K, Aryal P, Ter Horst KW, Cakir H, Romijn JA, Ackermans MT, Janssen IM, Berends FJ, van de Laar AW, Houdijk AP, Kahn BB, Serlie MJ. RBP4 increases lipolysis in human adipocytes and is associated with increased lipolysis and hepatic insulin resistance in obese women. FASEB J. 2020 May;34(5):6099-6110. doi: 10.1096/fj.201901979RR. Epub 2020 Mar 13. PMID: 32167208; PMCID: PMC7317205.