Catalog #80733

V.2_2

Human Retinol Binding Protein (RBP4) ELISA Kit Instructions

For the quantitative determination of retinol binding protein (RBP4) in human serum, plasma, and urine

Catalog #80733
96 Assays

For research use only. Not for use in diagnostic procedures.

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A. Intended Use

The Human Retinol Binding Protein (RBP4) ELISA kit is for the quantitative determination of retinol binding protein in human serum, plasma, and urine. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY, and it is not intended for use in diagnostic procedures.

B. Introduction

Retinol-binding protein (RBP4) is a carrier protein that binds retinol, a form of vitamin A. It plays a key role during pregnancy for the regulation and transport of retinol throughout the body.

C. Principle of the Assay

The Human Retinol Binding Protein (RBP4) ELISA kit is a double antibody sandwich ELISA. An unknown amount of retinol binding protein (RBP4) present in the sample binds with anti-RBP4 antibodies adsorbed to the surface of the microplate. After washing to remove unbound proteins, HRP-conjugated anti-RBP4 antibodies are added and form a complex with the retinol binding protein complex present in the wells. TMB substrate is then added to measure the concentration of retinol binding protein (RBP4) present by colorimetric detection.

D. Kit Storage

1. Upon receipt of the Human Retinol Binding Protein (RBP4) ELISA kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
MIC	Antibody-coated Microplate (12 x 8)	1 pack
CAL	Calibrator (Lyophilized)	1 vial
DIL	Diluent (5X Concentrate)	1 x 50 mL
HRP AB	HRP-Antibody (100X Concentrate)	1 vial/150 µL
WASH	Wash Buffer (20X Concentrate)	1 x 50 mL
SUB	Substrate Solution	1 x 12 mL
STOP	Stop Solution	1 x 12 mL

E.2. Materials required but not provided

Micropipettes and disposable tips
Distilled or deionized water
Polypropylene microtubes
Microplate reader (capable of reading A_{450} and A_{630} values)

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and

eyes. In case of contact with eyes or skin, flush immediately with water and contact a medical professional.
2. Some assay components may contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.

G. Maximizing Kit Performance

1. Given the sample volumes required (5 µL), pipetting should be done as carefully as possible. A high quality 10 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each standard and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. The samples should be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles. Samples with excessive hemolysis should not be used. *Note: Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.*

I. Assay Procedure

I.1. Preparation of reagents

1. Antibody-coated microplate
   Provided as ready to use. Protect from moisture.
2. Calibrator
   The calibrator is provided in lyophlized form and needs to be reconstituted with 1 mL of distilled or deionized water. Once reconstituted, the calibrator is a 2.0 µg/mL stock solution. The reconstituted calibrator should be stored frozen for future use and aliquoted in appropriate volumes prior to being frozen. Working standards should be prepared immediately prior to use as described in Section I.2. The working standard concentrations are 0, 1.56, 3.125, 6.25, 12.5, 25, 50, and 100 ng/mL.
3. Diluent (5X Concentrated)
   The diluent has to be diluted 1:5 with distilled or deionized water prior to use. For example, 50 mL of diluent must be diluted with 200 mL of distilled or deionized water. Diluent is stable for at least one week after dilution.
4. HRP-Antibody (100X Concentrated)
   The HRP-Antibody has to be diluted 1:100 with 1X Diluent prior to use. For each test strip, mix 10 µL of HRP-Antibody with 990 µL of 1X Diluent. Mix uniformly, but gently. Avoid foaming. The HRP-Antibody is stable for up to 1 hour when stored in the dark. Accordingly, HRP-Antibody should be prepared only as needed just prior to use.
5. Wash Buffer (20X Concentrated)
   The wash buffer has to be diluted 1:20 with distilled or deionized water prior to use. For example, 50 mL of wash buffer must be diluted with 950 mL of

distilled or deionized water. Wash buffer is stable for at least one week after dilution if stored at room temperature or at 2-8°C.

6. Substrate Solution
   Provided as ready to use.
7. Stop Solution
   Provided as ready to use.

I.2. Preparation of working standards

1. Pipette 950 µL of 1X diluent and 50 µL of the reconstituted calibrator (2.0 µg/mL) into a polypropylene microtube labeled 100 ng/mL, and mix thoroughly.
2. Dispense 300 µL of 1X diluent into six polypropylene microtubes labeled 1.56, 3.125, 6.25, 12.5, 25, and 50 ng/mL.
3. Dispense 300 µL of the 100 ng/mL standard into the 50 ng/mL microtube, and mix thoroughly.
4. Dispense 300 µL of the 50 ng/mL standard into the 25 ng/mL microtube, and mix thoroughly.
5. Dispense 300 µL of the 25 ng/mL standard into the 12.5 ng/mL microtube, and mix thoroughly.
6. Dispense 300 µL of the 12.5 ng/mL standard into the 6.25 ng/mL microtube, and mix thoroughly.
7. Dispense 300 µL of the 6.25 ng/mL standard into the 3.125 ng/mL microtube, and mix thoroughly.
8. Dispense 300 µL of the 3.125 ng/mL standard into the 1.56 ng/mL microtube, and mix thoroughly.
9. Dispense 500 µL of 1X diluent into one polypropylene microtube labeled 0 ng/mL. You should now have working standards of 0, 1.56, 3.125, 6.25, 12.5, 25, 50, and 100 ng/mL.

Please note: Working standards should be prepared immediately prior to use.

I.3. Dilution of samples

Samples need to be diluted with 1X diluent for use with the assay.

Serum/Plasma Samples: A sample dilution of 1:2000 using 5 µL of sample is generally suitable. To prepare the 1:2000 dilution, first mix 5 µL of sample with 495 µL of 1X Diluent to achieve a 1:100 dilution. Next, transfer 20 µL of the 1:100 to 380 µL of 1X Diluent to achieve a 1:2000 dilution. Mix thoroughly.

Urine Samples: A sample dilution of 1:20 using 20 µL of sample is generally suitable. To prepare the 1:20 dilution, mix 20 µL of sample with 380 µL of 1X Diluent. Mix thoroughly.

Since retinol binding protein levels can vary, dilution ratios may need to be adjusted as necessary.

I.4. Assay procedure

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. In each well, add 100 µL of diluted sample or working standard.
2. Incubate plate for 1 hour at room temperature. Keep plate covered and level.

3. Aspirate well contents and wash four times using 300 µL of 1X Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
4. In each well, add 100 µL of diluted HRP-Antibody.
5. Incubate plate for 10 mins at room temperature. Keep plate covered, in the dark, and level.
6. Aspirate well contents and wash four times using 300 µL of 1X Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
7. Add 100 µL of the Substrate Solution in each well.
8. Incubate plate for 10 mins in the dark, level, and at room temperature.
9. After 10 mins, stop the reaction by adding 100 µL of Stop Solution.
10. Measure absorbance within 30 minutes using a plate reader (measure A_{450} values and subtract A_{630} values).

I.5. Determining the retinol binding protein (RBP4) concentration

1. Using computer software, construct the retinol binding protein (RBP4) calibration curve by plotting the blank corrected mean absorbance value for each standard on the Y axis versus the corresponding RBP4 concentration on the X axis. Blank corrected values are determined by subtracting the mean absorbance value of the blank from the mean absorbance value for each standard. A four parametric logistic (4-PL) curve fit or second order polynomial (quadratic) are suitable for the evaluation. ***Note:*** *A calibration curve should be plotted every time the assay is performed.*

2. Human retinol binding protein (RBP4) concentrations in the samples are interpolated using the calibration curve and blank corrected mean absorbance values for each sample. For diluted samples, the values obtained must be multiplied by the dilution factor (ie. 2000) to obtain the final retinol binding protein (RBP4) concentration (expressed in ng/mL).
   Note: *Samples with high retinol binding protein (RBP4) concentrations (ie. fall above the range of the assay) should be further diluted and rerun.*

J. Performance characteristics

J.1. Assay range

The Human Retinol Binding Protein (RBP4) ELISA Kit has an assay range from 1.56 - 100 ng/mL.

J.2. Sensitivity

The assay has an average sensitivity of 1.2 ng/mL.

Warranty

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