RENBP (Human) ELISA Kit

Catalog Number KA1838

96 assays

Version: 01

Intended for research use only

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Introduction and Background

A. Overview

Retinol-binding protein (RENBP; RBP) is a transport protein that acts by solubilizing and protecting its labile ligands in aqueous spaces. It also has diverse and specific functions in regulating the disposition, metabolism and activities of retinoids (1). Retinol-binding protein is the specific plasma carrier of retinol, and encharged of the vitamin transport from the liver to target cells (2). Lower serum RENBP level associates with diarrhea (3). High level of RENBP in urine could be a good indicator of renal damage (4), microvascular complications with type-2 diabetes mellitus (5).

B. Test Principle

The RENBP (Human) ELISA Kit is designed for detection of human RENBP in plasma and serum. This assay employs a quantitative competitive enzyme immunoassay technique that measures RENBP in less than 3 hours. An antibody specific for RENBP has been pre-coated onto a microplate. RENBP in standards and samples is competed with a biotinylated RENBP sandwiched by the immobilized antibody and streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

C. Notice for Application of Kit

- ✓ **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated-protein, and SP conjugate) as instructed, prior to running the assay.**
- ✓ **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- ✓ **Spin down the SP conjugate vial before opening and using contents.**
- ✓ This kit is for research use only.
- ✓ The kit should not be used beyond the expiration date.
- ✓ The Stop Solution is an acid solution.

Material and Method

A. List of component

- ✓ **RBP Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human RBP.
- ✓ **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- ✓ **RBP Standard:** Human RBP in a buffered protein base (100 µg, lyophilized).
- ✓ **Biotinylated RBP:** 1 vial, lyophilized.
- ✓ **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 mL).
- ✓ **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 mL, 1 bottle).
- ✓ **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µL).
- ✓ **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 mL).
- ✓ **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 mL).

B. Additional Required Materials But Not Provided

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channel).
- ✓ Deionized or distilled reagent grade water.

C. Sample Collection, Preparation and Storage

- ✓ **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 xg for 10 minutes and assay. Dilute samples 1:20 into MIX Diluent. The undiluted samples can be stored at -20℃ or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)
- ✓ **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 xg for 10 minutes. Remove serum and assay. Dilute samples 1:20 into MIX Diluent. The undiluted samples can be stored at -20℃ or below for up to 3 months. Avoid repeated freeze-thaw cycles.

D. Reagent Preparation

- ✓ Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- ✓ **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2 - 8℃.
- ✓ **RBP Standard:** Reconstitute the 100 µg of human RBP Standard with 2 mL of MIX Diluent to generate a standard solution of 50 µg/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (50 µg/mL) 1:4 with MIX Diluent to produce 12.5, 3.125, 0.781, and 0.195 µg/mL. MIX Diluent serves as

the zero standard (0 µg /ml). Any remaining solution should be frozen at -20℃.

Standard Point	Dilution	RENBP (µg/mL)
P1	1 part stock (4 µg/mL)	4.00
P2	1 part P1 + 1 part EIA Diluent	2.00
P3	1 part P2 + 1 part EIA Diluent	1.00
P4	1 part P3 + 1 part EIA Diluent	0.500
P5	1 part P4 + 1 part EIA Diluent	0.250
P6	1 part P5 + 1 part EIA Diluent	0.125
P7	1 part P6 + 1 part EIA Diluent	0.0625
P8	EIA Diluent	0.0000

- ✓ **Biotinylated RBP (1x):** Dilute Biotinylated RBP with 4 mL MIX Diluent to produce a working solution. Allow the biotin to sit for 10 minutes with gentle agitation prior to use. Any remaining solution should be frozen at -20℃.
- ✓ **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- ✓ **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20℃.

E. Stability and storage

- ✓ Store components of the kit at 2-8℃ or -20℃ upon arrival up to the expiration date.
- ✓ Store SP Conjugate at -20℃.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8℃.
- ✓ Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- ✓ Diluent (1x) may be stored for up to 1 month at 2-8℃.
- ✓ Store Standard and Biotinylated Protein at 2-8℃ before reconstituting with Diluent and at -20℃ after reconstituting with Diluent.

F. Protocol

- ✓ Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30℃).
- ✓ Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- ✓ Add 25 µL of standard and/or sample per well, and immediately add 25 µL of **Biotinylated RBP** to each well (on top of the standard or sample). Cover wells with a sealing tape and incubate for two hours at room temperature. Start the timer after the last sample addition.
- ✓ Wash five times with 200 µL of **Wash Buffer** manually. Invert the plate each time and decant the

contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µL of **Wash Buffer** and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.

- ✓ Add 50 µL of **Streptavidin-Peroxidase Conjugate** to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- ✓ Wash the microplate as described above.
- ✓ Add 50 µL of **Chromogen Substrate** per well and incubate for about 15 minutes or until the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- ✓ Add 50 µL of **Stop Solution** to each well. The color will change from blue to yellow.
- ✓ Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

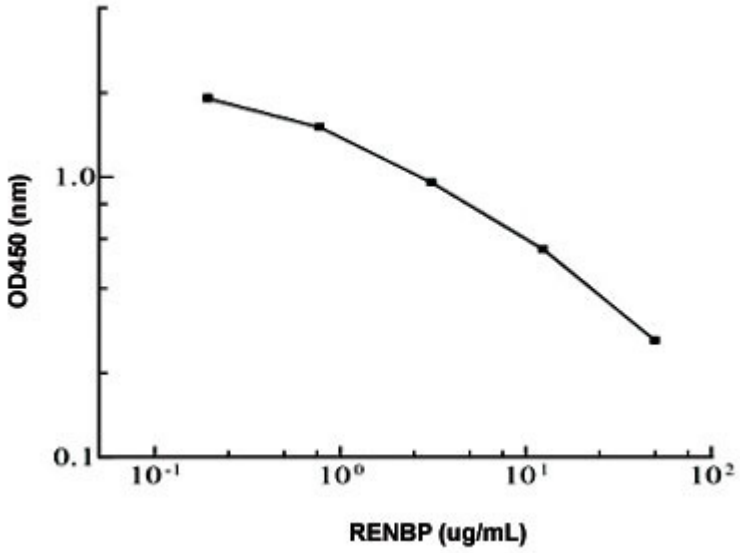
Result

A. Data analysis

- ✓ Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- ✓ To generate a Standard Curve, plot the graph using the standard concentrations on the X-axis and the corresponding mean 450 nm absorbance on the Y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- ✓ Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

B. Standard Curve

- ✓ The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- ✓ The minimum detectable dose of RBP is typically ~0.1 µg/mL.
- ✓ Intra-assay and inter-assay coefficients of variation were 4.5 % and 7.2% respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:10	88%	89%
1:20	94%	93%
1:40	93%	87%

Recovery

Standard Added Value	0.5 – 5 µg/mL
Recovery %	81-111%
Average Recovery %	97 %

References

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(2). Bellovino D et. al (2003) Mol Aspects Med. 24(6):411-20

(3). Mitra AK et. al (2002) J Health Popul Nutr. 20(1): 12-7

(4). Corso A et. al. (1999) Ann Hematol. 78(8): 371-5

(5). Hong CY et. al (2000) J Diabetes Complications 14(5):259-65