Datasheet for ABIN612762

Retinol-Binding Protein ELISA Kit

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Overview

Quantity:	96 tests
Target:	Retinol-Binding Protein (RBP)
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	0.007 µg/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human RBP ELISA kit is designed for detection of human RBP in urine, milk, and cell culture supernatants
Brand:	AssayMax
Sample Type:	Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	10% FBS in culture media will not affect the assay.
Components:	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 (3 µg, lyophilized). 1 Biotinylated RBP Antibody (50x): A 50-fold biotinylated polyclonal antibody against RBP (140µl). EIA Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A 20-fold

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	concentrated buffered surfactant (30 ml, 2 bottles). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80µl). 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).
Material not included:	Microplate reader capable of measuring absorbance at 450 nm. Pipettes (1-20 µL, 20-200 µL, 200-1000µLand multiple channel). Deionized or distilled reagent grade water.

Target Details

Target:	Retinol-Binding Protein (RBP)
Abstract:	RBP Products
Background:	Retinol-binding protein (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. Retinol-binding protein is the specific plasma carrier of retinol, and encharged of the vitamin transport from the liver to target cells. Lower serum RBP level associates with diarrhea. High level of RBP in urine could be a good indicator of renal damage , microvascular complications with type-2 diabetes mellitus.

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Sample Volume:	50 µL
Assay Time:	< 4 h
Plate:	Pre-coated
Protocol:	This assay employs a quantitative sandwich enzyme immunoassay technique that measures RBP in less than 4 hours. A polyclonal antibody specific for RBP has been pre-coated onto a microplate. RBP in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for RBP, which `is recognized by a 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.
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. EIA Diluent Concentrate (10x): Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. RBP Standard: Reconstitute the 3 g of human RBP Standard

	with 3 ml of EIA Diluent to generate a stock solution of 1 g/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the 2 Standard solution (1 g/ml) 1:2 with equal volume of EIA Diluent to produce 0.5, 0.25, 0.125, 0.063, 0.031, 0.016 and 0.008 g/ml. EIA Diluent serves as the zero standard (0 g /ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [RBP] (g/ml) 1 part Standard (1 g/ml) + 1 part EIA Diluent P1 0.500 P2 1 part P1 + 1 part EIA Diluent 0.250 P3 1 part P2 + 1 part EIA Diluent 0.125 P4 1 part P3 + 1 part EIA Diluent 0.063 P5 1 part P4 + 1 part EIA Diluent 0.031 P6 1 part P5 + 1 part EIA Diluent 0.016 P7 1 part P6 + 1 part EIA Diluent 0.008 P8 EIA Diluent 0.000 Biotinylated RBP Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with EIA Diluent. Any remaining solution should be frozen at -20°C. 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 EIA Diluent. Any remaining solution should be frozen at -20°C.
Sample Collection:	Cell Culture Supernatants: Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles. Urine: Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
Assay Procedure:	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 °C). 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 50 µL of Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. 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 Biotinylated RBP Antibody to each well and incubate for one hour. Wash a microplate as described above. Add 50 µL of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash a microplate as described above. Add 50 µL of Chromogen Substrate per well and incubate for about 12 minutes or till the

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	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 3 unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.
Calculation of Results:	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. Standard Curve The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.
Assay Precision:	Intra-assay and inter-assay coefficients of variation were 4.5 % and 7.2% respectively.
Restrictions:	For Research Use only

Handling

Handling Advice:	Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated- antibody, 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 and the biotinylated-antibody vial before opening and using contents. The kit should not be used beyond the expiration date.
Storage:	4 °C/-20 °C
Storage Comment:	Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date. Store SP Conjugate and Biotinylated Antibody at -20°C Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C 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°C. Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.