Datasheet for ABIN5564583

RBP4 ELISA Kit

1 Image

Go to Product page

Overview

Quantity:	96 tests
Target:	RBP4
Reactivity:	Dog
Method Type:	Sandwich ELISA
Detection Range:	0.781-50 ng/mL
Minimum Detection Limit:	0.781 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax™ Canine RBP4 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of canine RBP4 in plasma, serum, urine, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay that measures canine RBP4 in less than 4 hours. A polyclonal antibody specific for canine RBP4 has been pre-coated onto a 96-well microplate with removable strips. Canine RBP4 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for canine RBP4, 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.
Brand:	AssayMax™
Sample Type:	Cell Culture Cells, Plasma, Serum, Urine
Analytical Method:	Quantitative

Product Details

Detection Method:	Colorimetric
Components:	Canine RBP4 Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against canine RBP4. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes, which can be cut to fit the format of the individual assay. Canine RBP4 Standard: Canine RBP4 in a buffered protein base (50 ng, lyophilized). Biotinylated Canine RBP4 Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against canine RBP4 (140 l). 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, 2 bottles). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80 l). 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 405 nm. Pipettes (1-20 µL, 20-200 µL, and multiple channel). Deionized or distilled reagent grade water Incubator (37 °C)

Target Details

Target:	RBP4
Alternative Name:	Retinol-Binding Protein 4 (RBP4) (RBP4 Products)
Background:	Serum retinol-binding protein (RBP4), secreted by the liver and adipocytes, is implicated in systemic insulin resistance. RBP4 transports retinol and circulates in the plasma by binding to the larger transthyretin (TTR) homotetramer, forming a protein complex that reduces renal clearance of RBP4. In insulin-resistant ob/ob mice, urinary fractional excretion of RBP4 was reduced, which is consistent with increased retention, while TTR level is elevated (1). RBP4 is encoded by the RBP4 gene which maps to chromosome 10q23-q24 (2, 3). Transgenic overexpression of human RBP4 or injection of recombinant RBP4 in normal mice causes insulin resistance. Conversely, genetic deletion of RBP4 enhances insulin sensitivity. Increasing serum RBP4 induces hepatic expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase and impairs insulin signaling in muscle tissue (4). Expression of RBP4 is induced in adipose tissue as a consequence of decreased glucose transporter GLUT4 expression.
Gene ID:	477775
UniProt:	F1Q4D9
Pathways:	Regulatory RNA Pathways, Positive Regulation of Peptide Hormone Secretion, Carbohydrate Homeostasis, Production of Molecular Mediator of Immune Response

Application Details

Assay Time:	4 h
Plate:	Pre-coated
Protocol:	• Step 1. Add 50 µL of Standard or Sample per well. Incubate 2 hours. • Step 2. Wash, then add 50 µL of Biotinylated Antibody per well. Incubate 1 hour. • Step 3. Wash, then add 50 µL of SP Conjugate per well. Incubate 30 minutes. • Step 4. Wash, then add 50 µL of Chromogen Substrate per well. Incubate 10 minutes. • Step 5. Add 50 µL of Stop Solution per well. Read at 450 nm immediately.
Reagent Preparation:	Freshly dilute all reagents and bring all reagents to room temperature before use. MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8 °C. Canine RBP4 Standard: Reconstitute the 50 ng of Canine RBP4 Standard with 1 mL of MIX Diluent to generate a 50 ng/mL standard stock solution. 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 stock solution (50 ng/mL) 1:2 with MIX Diluent to produce 25, 12.5, 6.25, 3.125, 1.563, and 0.781 ng/mL solutions. MIX Diluent serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at -20 °C and used within 30 days. Standard Point Dilution [Canine RBP4] (ng/mL) P1 1 part Standard (50 ng/mL) 50.00 P2 1 part P1 + 1 part MIX Diluent 25.00 P3 1 part P2 + 1 part MIX Diluent 12.50 P4 1 part P3 + 1 part MIX Diluent 6.250 P5 1 part P4 + 1 part MIX Diluent 3.125 P6 1 part P5 + 1 part MIX Diluent 1.563 P7 1 part P6 + 1 part MIX Diluent 0.781 P8 MIX Diluent 0.000 Biotinylated Canine RBP4 Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20 °C. Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. 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 °C.
Sample Collection:	Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes. Dilute samples 1:5000 into MIX Diluent. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant). Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes, and remove serum. Dilute samples 1:5000 into MIX Diluent and assay. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for

	10 minutes. Dilute urine 1:4 into MIX Diluent and assay. Samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20 °C or below. Avoid repeated freeze-thaw cycles. Refer to Sample Dilution Guidelines below for further instruction. Guidelines for Dilutions of 1:100 or Greater (for reference only, please follow the insert for specific dilution suggested) 1:100 1:10000 A) 4 µL sample: 396 µL buffer(100x) = 100 fold dilution Assuming the needed volume is less than or equal to 400 µL. A) 4 µL sample : 396 µL buffer (100x) B) 4 µL of A : 396 µL buffer (100x) = 10000 fold dilution Assuming the needed volume is less than or equal to 400 µL. 1:1000 1:100000 A) 4 µL sample : 396 µL buffer (100x) B) 24 µL of A : 216 µL buffer (10x) = 1000 fold dilution Assuming the needed volume is less than or equal to 240 µL. A) 4 µL sample : 396 µL buffer (100x) B) 4 µL of A : 396 µL buffer (100x) C) 24 µL of B : 216 µL buffer (10x) = 100000 fold dilution Assuming the needed volume is less than or equal to 240 µL. 4
Assay Procedure:	Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25 °C). Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch 5 securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50 l of Canine RBP4 Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition. Wash five times with 200 l of Wash Buffer manually. Invert the plate each time and decant the contents, hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 l of Wash Buffer and then invert the plate, decanting the contents, hit 4-5 times on absorbent material to completely remove the liquid. Add 50 l of Biotinylated Canine RBP4 Antibody to each well and incubate for 1 hour. Wash the microplate as described above. Add 50 l of Streptavidin-Peroxidase Conjugate per 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 l of Chromogen Substrate per well and incubate for 10 minutes or till 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 l 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.

Application Details

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 (OD) 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.
Restrictions:	For Research Use only

Handling

Handling Advice:	This product is for Research Use Only and is Not For Use In Diagnostic Procedures. Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay. 2 Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. 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 Stop Solution is an acidic solution. The kit should not be used beyond the expiration date.
Storage:	4 °C,-20 °C
Storage Comment:	Upon arrival, immediately store components of the kit at recommended temperatures 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. Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator. Diluent (1x) may be stored for up to 30 days at 2-8°C. Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent. 3

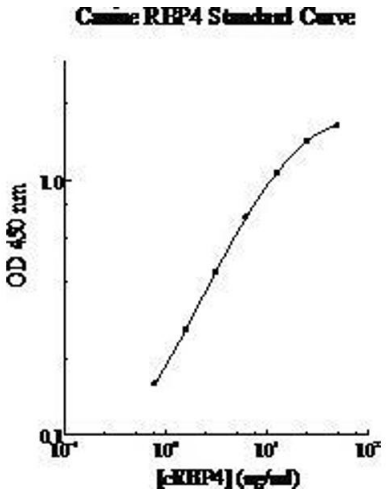

Image 1.