Datasheet for ABIN5564617

FABP4 ELISA Kit

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Overview

Quantity:	96 tests
Target:	FABP4
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.488-125 ng/mL
Minimum Detection Limit:	0.488 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax™ Human FABP4 ELISA (Enzyme-Linked Immunosorbent Assay) Kit is designed for detection of FABP4 in human plasma, serum, urine, milk, CSF, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human FABP4 in approximately 5 hours. A polyclonal antibody specific for human FABP4 has been pre-coated onto a 96-well microplate with removable strips. FABP4 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human FABP4, which is recognized by a streptavidin-peroxidase (SP) conjugate. All unbound material is 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, Cerebrospinal Fluid, Milk, Plasma, Serum, Urine
Analytical Method:	Quantitative

Product Details

Detection Method:	Colorimetric
Components:	Human FABP4 Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human FABP4. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. Human FABP4 Standard: Human FABP4 in a buffered protein base (500 ng, lyophilized, 2 vials). Biotinylated Human FABP4 Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against human FABP4 (120 l). EIA Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (20 ml). Standard Diluent (1x): A buffered protein base with stabilizer (2 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml, 2 bottles). SP Conjugate (100x): A 100-fold concentrate (80 l). Chromogen Substrate (1x): A stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml). Stop Solution (1x): A 0.5 N hydrochloric acid solution 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:	FABP4
Alternative Name:	Fatty Acid-Binding Protein 4 (FABP4) (FABP4 Products)
Background:	Fatty acid-binding protein 4 (FABP4), also called adipocyte lipid-binding protein, belongs to a family of mall cytoplasmic intracellular lipid-binding proteins involved in the transport and storage of lipids in adipocytes and macrophages. It consists of 132 amino acid residues and has a molecular mass of 14 - 15 kDa (1). FABP4 binds fatty acids, retinoids, and other hydrophobic ligands and then transports them to the nucleus, where the FABP4/fatty acid complex activates peroxisome proliferator-activated receptor gamma (PPAR- gamma). FABP4 and PPAR-gamma work together to influence a biologic pathway affecting insulin sensitivity and body composition (2). It forms a physical complex with the hormone-sensitive lipase, activating the enzyme to facilitate intracellular trafficking of hydrophobic lipids (3). FABP4 serves as a locus of integration for metabolic and inflammatory pathways.
Gene ID:	2167
UniProt:	P15090
Pathways:	Brown Fat Cell Differentiation

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 2 hours. • 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 20 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. EIA Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 10-fold with reagent grade water to produce a 1x solution. Store for up to 30 days at 2-8 °C. Human FABP4 Standard: Reconstitute the Human FABP4 Standard (500 ng) with 0.5 mL of Standard Diluent to generate a 1000 ng/mL standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. From the standard stock solution (1000 ng/mL), dilute 8-fold with EIA Diluent to produce a 125 ng/mL standard working solution. Prepare duplicate or triplicate standard points by serially diluting the standard working solution (125 ng/mL) 4-fold with EIA Diluent to produce 31.25, 7.813, 1.953, and 0.488 ng/mL solutions. EIA Diluent serves as the zero standard (0 ng/mL). Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20 °C and used within 48 hours. Standard Point Dilution [FABP4] (ng/mL) P1 1 part Standard (1000 ng/mL) + 7 parts EIA Diluent 125 P2 1 part P1 + 3 parts EIA Diluent 31.25 P3 1 part P2 + 3 parts EIA Diluent 7.813 P4 1 part P3 + 3 parts EIA Diluent 1.953 P5 1 part P4 + 3 parts EIA Diluent 0.488 P6 EIA Diluent 0.0 5 Biotinylated Human FABP4 Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with EIA Diluent to produce a 1x solution. The undiluted antibody should be stored 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 20-fold with reagent grade water to produce a 1x solution. SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with EIA Diluent to produce a 1x solution. The undiluted conjugate should be stored 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 and collect plasma. A 2-fold sample dilution is suggested into EIA Diluent, however, user should determine optimal dilution factor depending on application needs. 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. A 2-fold sample dilution is suggested into EIA Diluent, however, user should determine optimal dilution factor depending on application needs. 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. The sample is suggested for use at 1x, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored 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. The sample is suggested for use at 1x, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. CSF: Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x g for 10 minutes. The sample is suggested for use at 1x, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80 °C for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes at 4 °C to remove debris and collect supernatants. Samples can be stored at -20 °C or below. Avoid repeated freeze-thaw cycles.
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 securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50 l of Human FABP4 Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. 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 Human FABP4 Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Wash the microplate as described above. Add 50 l of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape 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 to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that

Application Details

	may have formed. Incubate for 20 minutes or until the optimal blue color density develops. Add 50 l of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. 6 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.
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 intended for use in diagnostic procedures. Prepare all reagents (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, the biotinylated antibody vial, and the standard diluent 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 Standard, SP Conjugate, and Biotinylated Antibody at -20°C. Store Microplate, Diluent Concentrate (10x), Standard Diluent (1x), 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. 3

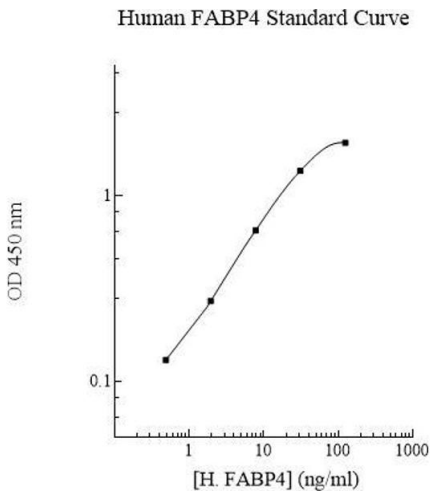

Image 1.

