Datasheet for ABIN5564628

VTA1 ELISA Kit

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Overview

Quantity:	96 tests
Target:	VTA1
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.391-25 ng/mL
Minimum Detection Limit:	0.391 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax™ Human VTA1 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human VTA1 in plasma, serum, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human VTA1 in less than 4 hours. A polyclonal antibody specific for human VTA1 has been pre-coated onto a 96-well microplate with removable strips. VTA1 in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for VTA1, which is recognized by a streptavidin-peroxidase 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, Plasma, Serum
Analytical Method:	Quantitative

Product Details

Detection Method:	Colorimetric
Components:	Human VTA1 Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human VTA1. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. Human VTA1 Standard: Human VTA1 in a buffered protein base (12.5 ng, lyophilized). Biotinylated Human VTA1 Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against VTA1 (120 l). EIA Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (20 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:	VTA1
Alternative Name:	VTA1 (VTA1 Products)
Background:	Vacuolar protein sorting-associated protein VTA1 homolog (VTA1), also known as dopamine-responsive gene 1 protein (DRG-1), LYST-interacting protein 5 (LIP5), and SKD1-binding protein 1 (SBP1), is predominantly a cytosolic protein. VTA1 consists of 307 amino acids with a predicted molecular mass of 34 kDa. It is involved in the endosomal multivesicular body (MVB) formation and trafficking. The MVB is an endosomal compartment that serves to sort membrane proteins destined for degradation or routing to the lysosome. These membrane proteins, such as stimulated growth factor receptors, lysosomal enzymes and lipids, are internalized into intraluminal vesicles that are generated by the invagination and scission from the limiting membrane of the endosome. The contents of the MVB are then transferred to lysosomes. VTA1 interacts with charged multivesicular body protein 5 (CHMP5) to form stable complexes in the cytoplasm, binds well to CHMP1B, CHMP2A, and CHMP3 proteins for MVB sorting, and is required for immunodeficiency virus type 1 HIV particle release (1-3).
Gene ID:	51534
UniProt:	Q9NP79

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 15 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 1:10 with reagent grade water. Store for up to 30 days at 2-8 °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 assay. 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 anticoagulant). Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes. Remove serum 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 the remaining samples 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 VTA1 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 Human VTA1 Antibody to each well and incubate for 1 hour. Wash the microplate as described above. Add 50 l 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. 5 Add 50 l of Chromogen Substrate per well and incubate for

Application Details

	15 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.
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. 2 Prepare all reagents (working diluent buffer, wash buffer, standard, 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 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. 3 Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

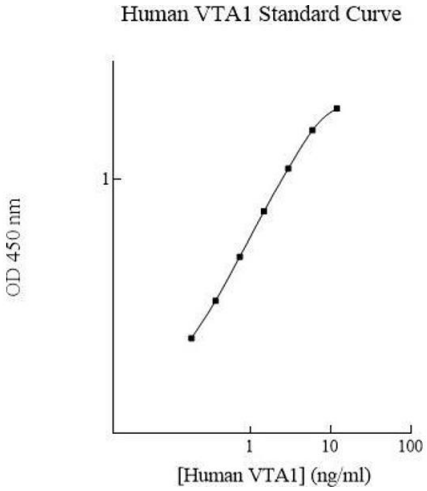

Image 1.

