Datasheet for ABIN5564560

AK4 ELISA Kit

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Overview

Quantity:	96 tests
Target:	AK4
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.313-20 ng/mL
Minimum Detection Limit:	0.313 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax™ Human Adenylate Kinase 4 (AK4) ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human AK4 in plasma, serum, saliva, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human AK4 in less than 4 hours. A polyclonal antibody specific for human AK4 has been pre- coated onto a 96-well microplate with removable strips. AK4 in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for AK4, 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, Cerebrospinal Fluid, Plasma, Saliva, Serum
Analytical Method:	Quantitative

Product Details

Detection Method:	Colorimetric
Components:	Human AK4 Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human AK4. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. Human AK4 Standard: Human AK4 in a buffered protein base (20 ng, lyophilized). Biotinylated Human AK4 Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against AK4 (120 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:	AK4
Alternative Name:	Adenylate Kinase 4 (AK 4) (AK4 Products)
Background:	Adenylate kinase 4 (AK4), also known as GTP:AMP phosphotransferase AK4, adenylate kinase 3-like and AK3L1, belongs to the adenylate kinase family. Nine different kinds of human AK isozymes have been reported. AK4 is localized to the mitochondrial matrix and has a high degree of sequence homology with AK3. It regulates the adenine and guanine nucleotide compositions within a cell by catalyzing the reversible transfer of phosphate group among these nucleotides. The 223-amino-acid protein efficiently phosphorylates AMP and dAMP using ATP as a phosphate donor, but phosphorylates only AMP when using GTP as a phosphate donor. It also displays broad nucleoside diphosphate kinase activity (1-2). AK4 is highly expressed in the kidney, moderately expressed in the heart and liver, and weakly expressed in the brain (3). AK4 is involved in stress, malignant progression, ATP regulation, hypoxia tolerance, drug resistance, and the regulation of mitochondrial activity (4-6).
Gene ID:	205
Pathways:	Nucleotide Phosphorylation, Ribonucleoside Biosynthetic Process

Application Details

Plate:	Pre-coated

Application Details

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. 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.
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 1:2 into MIX Diluent or within the range of 1x - 4x, and assay. Depending on application needs, user should determine proper dilutions. 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 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 1:2 into MIX Diluent or within the range of 1x - 4x, and assay. Depending on application needs, user should determine proper dilutions. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Saliva: Collect saliva 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. 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 AK4 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 AK4 Antibody to each well and incubate for 1 hour. Wash the microplate as described above. 5 Add 50 l of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn

Application Details

	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 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. 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

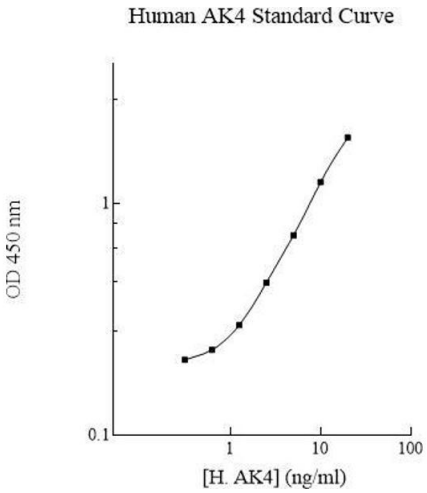

Image 1.

