Datasheet for ABIN612711

HSPD1 ELISA Kit

1 Image | 1 Publication

Go to Product page

Overview

Quantity:	96 tests
Target:	HSPD1
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	2.5-80 ng/mL
Minimum Detection Limit:	2.5 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Heat Shock Protein 60 (HSP60) ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human HSP60 in plasma, serum, cell culture lysates, and tissue samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human HSP60 in less than 5 hours. A polyclonal antibody specific for human HSP60 has been pre-coated onto a 96-well microplate with removable strips. HSP60 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for HSP60, 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, Tissue Lysate
Analytical Method:	Quantitative

Product Details

Detection Method:	Colorimetric
Components:	Human HSP60 Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human HSP60. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. Human HSP60 Standard: Human HSP60 in a buffered protein base (80 ng, lyophilized, 2 vials). Biotinylated Human HSP60 Antibody (80x): A 80-fold concentrated biotinylated polyclonal antibody against HSP60 (75 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 concentrated (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:	HSPD1
Alternative Name:	Heat Shock Protein 60 (HSP60) (HSPD1 Products)
Background:	Heat shock protein of 60 kDa (HSP60) is a mitochondrial chaperonin involved in folding, assembly, and transport of newly imported protein from cytoplasm into mitochondria in an ATP-mediated reaction (1-3). Human HSP60 contains 573 amino acids and is related to the bacteria groEL protein. HSP60 is located in the mitochondria and cytoplasm, the cell surface, the extracellular space, and the peripheral blood (4, 5). Under dehydration conditions, the cytoplasmic HSP60 is quickly imported into the mitochondria by cytoplasmic HSP70 (6). Extracellular HSP60 mediates apoptosis via Toll-like receptors (7). An HSP60 defect can cause neurodegenerative pathologies (8).
Gene ID:	3329
UniProt:	P10809
Pathways:	Activation of Innate immune Response, Regulation of Leukocyte Mediated Immunity, Positive Regulation of Immune Effector Process, Production of Molecular Mediator of Immune Response, Positive Regulation of Endopeptidase Activity

Application Details

Sample Volume:	50 µL
Assay Time:	5 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 12 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. Store samples 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. Collect the sample and assay. Store samples at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Lysates: Place the cell culture dish in ice and wash the cells with ice-cold PBS. Drain the PBS, then add ice-cold lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na2EDTA, 1 mM EGTA, 1 % Triton, 0.1 mM PMSF, 1 µg/mL leupeptin, 1 µg/mL aprotinin, and 1 µg/mL pepstatin). Scrape adherent cells off the dish and transfer the cell suspension into a pre-cooled microfuge tube. Maintain constant agitation for 30 minutes at 4 °C. Centrifuge in a microcentrifuge at 4 °C. Collect fresh cell lysates. The undiluted samples can be stored at -20 °C or below. Tissue: Extract tissue samples with 50 mM phosphate-buffered saline (pH 7.4) containing 1 % Triton X-100 and centrifuge at 14000 x g for 20 minutes. Collect the supernatant and measure the protein concentration. The undiluted samples can be stored at -20 °C or below.
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 HSP60 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

Application Details

	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 HSP60 Antibody to each well and incubate for 2 hours. 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 12 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.
Assay Precision:	Intra-assay and inter-assay coefficients of variation were 4.8% and 7.2% respectively.
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. 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. 2 The Stop Solution is an acidic solution. The kit should not be used beyond the expiration date.
Storage:	4 °C/-20 °C

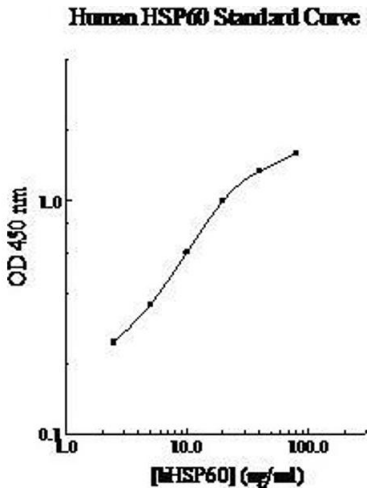
Handling

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. Fresh standard should be reconstituted the day the assay is run.

Publications

Product cited in:	Pasella, Pinna, Deiana, Baralla, Dore, Mannu, Canu, Sotgiu, Zinellu, Mangoni, Sotgia, Carru, Deiana: "Plasma concentrations of transthyretin in older Sardinians including centenarians." in: **Aging clinical and experimental research**, (2015) (PubMed). Murakami, Sango, Watabe, Niimi, Takaku, Li, Yamamura, Sunada: "Schwann cells contribute to neurodegeneration in transthyretin amyloidosis." in: **Journal of neurochemistry**, Vol. 134, Issue 1, pp. 66-74, (2015) (PubMed).

Images



ELISA

Image 1.