Datasheet for ABIN612709

HSP27 ELISA Kit

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Overview

Quantity:	96 tests
Target:	HSP27
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	0.3 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Hsp27 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human Hsp27 in plasma, serum, milk, tissue extract, and cell culture samples
Brand:	AssayMax
Sample Type:	Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	Human Hsp27 Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Hsp27. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. Human Hsp27 Standard: Human Hsp27 in a buffered protein base (160 ng, lyophilized). Biotinylated Hsp27 Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against Hsp27 (80µl). 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).

Product Details

	Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrated (80µl). 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 450 nm. Pipettes (1-20 µL, 20-200 µL, 200-1000µLand multiple channel). Deionized or distilled reagent grade water

Target Details

Target:	HSP27
Alternative Name:	Heat Shock Protein 27, HSP27 (HSP27 Products)
Background:	Heat shock proteins are molecular chaperones that have an ability to protect proteins from damage induced by environmental factors such as free radicals, heat, ischaemia and toxins, allowing denatured proteins to adopt their native configuration. Heat shock protein-27 (Hsp27) is a member of the small Hsp (sHsp) family of proteins, and has a molecular weight of approximately 27 KDa. In addition to its role as a chaperone, it has also been reported to have many additional functions. These include effects on the apoptotic pathway, cell movement and embryogenesis. It is suggested that Hsp27 may play a key role in resistance to doxorubicin-induced cardiac dysfunction, and lower lymphocyte Hsp27 levels might be associated with an increased risk of lung cancer. HSP27 expression is enhanced in target tissues of diabetic microvascular complications, and changes in circulating serum Hsp27 levels (sHSP27) have been reported in patients with macrovascular disease.
Pathways:	VEGF Signaling

Application Details

Sample Volume:	50 µL
Assay Time:	< 5 h
Plate:	Pre-coated
Protocol:	This assay employs a quantitative sandwich enzyme immunoassay technique that measures human Hsp27 in less than 5 hours. A polyclonal antibody specific for human Hsp27 has been pre-coated onto a 96-well microplate with removable strips. Hsp27 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for Hsp27, 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.
Reagent Preparation:	Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. EIA Diluent Concentrate (10x): Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Standard Curve: Reconstitute the 160 ng of Hsp27 Standard with 2 ml of EIA Diluent to generate a solution of 80 ng/ml. 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 solution (80 ng/ml) 1:4 with EIA Diluent to produce 20, 5, 1.25 and 0.32 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [Hsp27] (ng/ml) P1 Standard (80 ng/ml) 80.00 P2 1 part P1 + 3 parts EIA Diluent 20.00 P3 1 part P2 + 3 parts EIA Diluent 5.00 P4 1 part P3 + 3 parts EIA Diluent 1.25 P5 1 part P4 + 3 parts EIA Diluent 0.32 P6 EIA Diluent 0.00 Biotin Hsp27 Antibody (100x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C. Wash Buffer Concentrate (20x): 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 EIA 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 2000 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 anticoagulant.) Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 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, 0.1mM PMSF, 1g/ml leupeptin, 1g/mL aprotinin, and 1g/mL pepstatin.). Scrape adherent cells off the dish and then transfer the 2 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 and assay. The undiluted samples can be stored at -20°C or below. Tissue: Extract tissue samples with 50 mM phosphate-buffered saline (pH7.4) containing 1% Triton x-100 and centrifuge at 14000x g for 20 min. Collect the supernatant, measure the protein concentration and assay. The undiluted samples can be stored at -20°C or below. Milk: Collect milk using sample tube. Centrifuge samples at 600 x g for 10 minutes. Milk dilution is suggested at 1:2 in MIx Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Application Details

Assay Procedure:	Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20 - 30 °C). Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50 µL of Hsp27 standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents, hit it 4-5 times on absorbent paper towel to completely remove the liquid. 3 If using a machine wash six times with 300 µL of Wash Buffer and then invert the plate, decant the contents, hit it 4-5 times on absorbent paper towel to completely remove the liquid. Add 50 µL of Biotinylated Hsp27 Antibody to each well and incubate for two hours. Wash the microplate as described above. Add 50 µL 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. Add 50 µL of Chromogen Substrate per well and incubate for about 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 µL 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 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 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. Standard Curve The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.
Assay Precision:	Intra-assay and inter-assay coefficients of variation were 4.6% and 7.3% respectively.
Restrictions:	For Research Use only

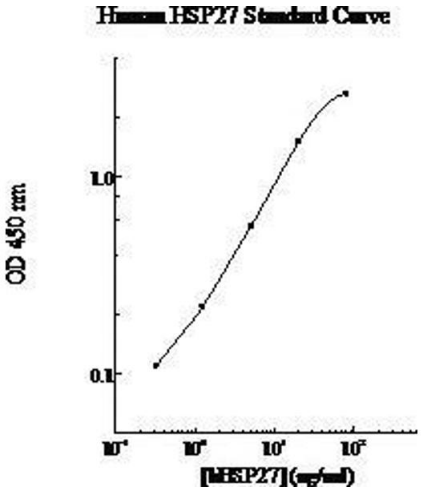
Handling

Handling Advice:	Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated- antibody, and

Handling

	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 protocol. 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. 1 The kit should not be used beyond the expiration date.
Storage:	4 °C/-20 °C
Storage Comment:	Store components of the kit at 2-8°C or -20°C upon arrival 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 Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator. Diluent (1x) may be stored for up to 1 month at 2-8°C. Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Images



ELISA

Image 1.