Datasheet for ABIN612710

SERPINH1 ELISA Kit

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Overview

Quantity:	96 tests
Target:	SERPINH1
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	1.5 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human HSP47 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human HSP47 in plasma, serum, tissue extract, and cell culture lysates
Brand:	AssayMax
Sample Type:	Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	Human Hsp47 Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Hsp47. 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 Hsp47 Standard: Human Hsp47 in a buffered protein base (50 ng, lyophilized). Biotinylated Hsp47 Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against Hsp47 (80 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).

Product Details

	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 450 nm. Pipettes (1-20 µL, 20-200 µL, 200-1000l and multiple channel). Deionized or distilled reagent grade water

Target Details

Target:	SERPINH1
Alternative Name:	Heat Shock Protein-47 (Hsp47) (SERPINH1 Products)
Target Type:	Viral Protein
Background:	Heat-shock protein of 47 kDa (Hsp47), also called serpin H1, clade H, member 1, collagen binding protein 1 and colligin, is a member of the serpin superfamily of serine proteinase inhibitors. Hsp47 is a 418 amino acids collagen-specific molecular chaperone involved in the collagen folding and secretion. It localizes to the endoplasmic reticulum lumen and its expression is induced by heat shock (1-2). In the sera of the rheumatic autoimmune and mixed connective tissue disease patients, HSP47 antigen and/or autoantibody levels were elevated. High expression of Hsp47 was observed in ulcerative colitis-associated carcinomas cell lines and tissues. Hsp47 is over-expressed in many fibrotic diseases such as systemic sclerosis, pulmonary fibrosis, liver cirrhosis, cicatricial pemphigoid, epidermolysis bullosa acquisita and keloids (5-9). Hsp47 appears as a potential biomarker or therapeutic target for the treatment of collagen-related fibrosis.

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 Hsp47 in less than 5 hours. A polyclonal antibody specific for human Hsp47 has been pre-coated onto a 96-well microplate with removable strips. Hsp47 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for Hsp47, 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 50 ng of Hsp47 Standard with 1 ml of EIA Diluent to generate a solution of 50 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 (50 ng/ml) 1:2 with EIA Diluent to produce 25, 12.5, 6.25, 3.125 and 1.565 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 [Hsp47] (ng/ml) P1 Standard (50 ng/ml) 50.00 P2 1 part P1 + 1 part EIA Diluent 25.00 P3 1 part P2 + 1 part EIA Diluent 12.50 P4 1 part P3 + 1 part EIA Diluent 6.250 P5 1 part P4 + 1 part EIA Diluent 3.125 P6 1 part P5 + 1 part EIA Diluent 1.565 P7 EIA Diluent 0.000 Biotin Hsp47 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 cell 2 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. Use undiluted samples or 1:2 diluted samples with EIA Diluent 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 and measure the protein concentration. Use undiluted samples or 1:2 diluted samples with EIA Diluent and assay. The undiluted samples can be stored at -20°C or below.

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 Hsp47 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 Hsp47 Antibody to each well and incubate for two hours. Wash a 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 a microplate as described above. Add 50 µL of Chromogen Substrate per well and incubate for about 20 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. 3 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 4.9% and 7.1% respectively.
Restrictions:	For Research Use only

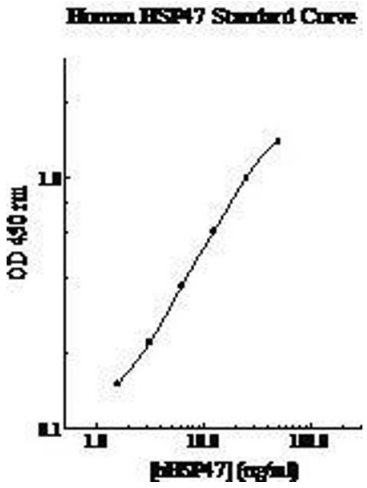
Handling

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. The kit should not be used beyond the expiration date. 1
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.