HSP27 (Human) ELISA Kit

Catalog Number KA0981

96 assays

Version: 05

Intended for research use only

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Introduction

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 and cell movement (1).

Principle of the Assay

The HSP27 (Human) ELISA Kit is designed for detection of human HSP27 in plasma, serum, milk, tissue extract, and cell culture samples. 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.

General Information

Materials Supplied

List of component

Component	Amount
Human HSP27 Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human HSP27.	96 (8x12) wells
Sealing Tapes: pressure-sensitive sealing tapes, which can be cut to fit the format of the individual assay.	3 slices
Human HSP27 Standard: Human HSP27 in a buffered protein base (lyophilized).	160 ng, 2 vials
Biotinylated Human HSP27 Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against HSP27	120 µL
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 µL
Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine	8 mL
Stop Solution: A 0.5 N hydroxychloric acid to stop the chromogen substrate reaction	12 mL

Storage Instruction

- ✓ 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 and Biotinylated Protein at 2-8°C before reconstituting with diluent and at -20°C after reconstituting with diluent.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channel).
- ✓ Deionized or distilled reagent grade water.

Precautions for Use

- ✓ 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 protein, 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 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.

Assay Protocol

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.
- Standard Curve: Reconstitute the 160 ng of Human Hsp27 Standard with 2 mL of EIA Diluent to generate an 80 ng/mL standard stock solution. 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 stock solution (80 ng/mL) 1:4 with EIA Diluent to produce 20, 5, 1.25 and 0.313 ng/mL solutions. EIA Diluent serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at -20°C and used within 5 days.

Standard Point	Dilution	[HSH27] (ng/mL)
P1	1 part Standard (80 ng/mL)	80.0
P2	1 part P1 + 3 parts EIA Diluent	20.0
P3	1 part P2 + 3 parts EIA Diluent	5.0
P4	1 part P3 + 3 parts EIA Diluent	1.25
P5	1 part P4 + 3 parts EIA Diluent	0.313
P6	EIA Diluent	0.000

- Biotinylated Human HSH27 Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with EIA Diluent. Any remaining solution should be frozen at -20°C.
- Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. 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 Preparation

- 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 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, 0.1mM PMSF, 1 µg/mL leupeptin, 1 µg/mL aprotinin, and 1 µg/mL pepstatin). Scrape

adherent cells off the dish and then 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 and assay. 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, 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 800 x g for 10 minutes. Milk dilution is suggested at 1:2 in EIA Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Assay Procedure

1. Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
2. 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.
3. Add 50 µL of Human HSH27 standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last sample addition.
4. 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 material to completely remove the liquid. 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 material to completely remove the liquid.
5. Add 50 µL of Biotinylated Human HSH27 Antibody to each well and incubate for 2 hours.
6. Wash a microplate as described above.
7. 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.
8. Wash the microplate as described above.
9. 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.
10. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.
11. 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 maybe generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

✓ Assay Summary

1. Add 50 µL of Standard or Sample per well. Incubate 2 hours.
2. Wash, then add 50 µL of Biotinylated Antibody per well. Incubate 2 hours.
3. Wash, then add 50 µL of SP Conjugate per well. Incubate 30 minutes.
4. Wash, then add 50 µL Chromogen Substrate per well. Incubate 15 minutes.
5. Add 50 µL of Stop Solution per well. Read at 450 nm immediately.

Data Analysis

Calculation of Results

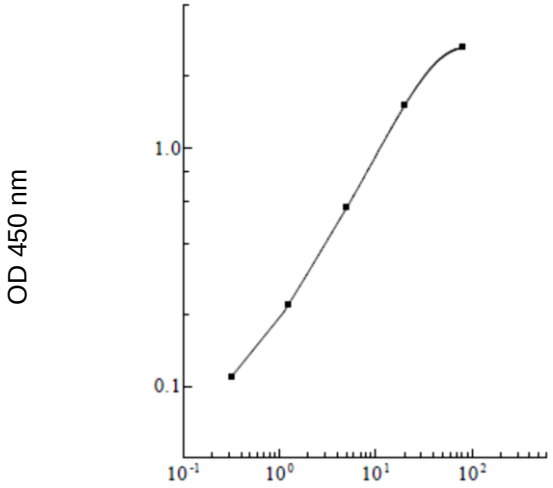
- ✓ Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- ✓ To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression 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.
- ✓ Typical Data

  The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	ng/mL	OD	Average OD
P1	80.0	2.185 2.189	2.187
P2	20.0	1.561 1.529	1.545
P3	5.0	0.800 0.784	0.792
P4	1.25	0.303 0.326	0.315
P5	0.313	0.134 0.137	0.135
P6	0.000	0.062 0.067	0.064

✓ Standard Curve

The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

✓ The minimum detectable dose of HSP27 as calculated by 2SD from the mean of a zero standard was established to be 0.18 ng/mL.

✓ Intra-assay precision was determined by testing replicates of three plasma samples in one assay.

✓ Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	3.7%	5.6%	2.1%	9.1%	7.8%	8.4%
Average CV (%)	3.8%			8.4%		

✓ Recovery

Standard Added Value	1.25 - 20 ng/mL
Recovery %	89 – 112 %
Average Recovery %	97 %

✓ Linearity

Milk samples were serially-diluted to test for linearity.

	Average Percentage of Expected Value (%)
Sample Dilution	Milk
No dilution	92%
1:2	98%
1:4	104%

✓ Cross-Reactivity

Species	Cross Reactivity (%)
Canine	20 %
Bovine	None
Monkey	50 %
Mouse	None
Rat	20 %
Swine	50 %
Rabbit	None

Resources

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots.
	Improper wash step	Check that the correct wash buffer is being used. Check that all wells are dry after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	Each step of the procedure should be performed uninterrupted.
	Omission of step	Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	Check pipette calibration. Check pipette for proper performance.
	Wash step was skipped	Consult the provided procedure for all wash steps.
	Improper wash buffer	Check that the correct wash buffer is being used.
	Improper reagent preparation	Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	Consult the provided procedure for correct incubation time.

Deficient Standard Curve Fit	Non-optimal sample dilution	Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. User should determine the optimal dilution factor for samples.
	Contamination of reagents	A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.

References

1. Ferns G *et al.* (2006) *Int J Exp Pathol.* 87(4):253-74.

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H