ab108862 – Heat Shock Protein 27 (HSP27) Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human Heat Shock Protein 27 (HSP27) in plasma, milk, serum, cell culture samples and tissue extract.

This product is for research use only and is not intended for diagnostic use.
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INTRODUCTION

1. BACKGROUND

Abcam’s Heat Shock Protein 27 (HSP27) Human in vitro ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of HSP27 levels in cell culture samples, milk, serum, plasma and tissue extract.

A HSP27 specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently a HSP27 specific biotinylated detection antibody is added and then followed by washing with wash buffer. Streptavidin-Peroxidase Conjugate is added and unbound conjugates are washed away with wash buffer. TMB is then used to visualize Streptavidin-Peroxidase enzymatic reaction. TMB is catalyzed by Streptavidin-Peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the amount of HSP27 captured in plate.

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. 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.
INTRODUCTION

2. ASSAY SUMMARY

Primary capture antibody
Prepare all reagents, samples and standards as instructed.

Sample
Add standard or sample to each well used. Incubate at room temperature.

Primary detector antibody
Wash and add prepared biotin antibody to each well. Incubate at room temperature.

Streptavidin Label
Wash and add prepared Streptavidin-Peroxidase Conjugate. Incubate at room temperature.

Substrate Colored product
Add Chromogen Substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.
3. **PRECAUTIONS**

Please read these instructions carefully prior to beginning the assay.

Modifications to the kit components or procedures may result in loss of performance.

4. **STORAGE AND STABILITY**

Store kit at 4°C immediately upon receipt, apart from the SP Conjugate & Biotinylated Antibody, which should be stored at -20°C.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. **MATERIALS SUPPLIED**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSP27 Microplate (12 x 8 well strips)</td>
<td>96 wells</td>
<td>4°C</td>
</tr>
<tr>
<td>HSP27 Standard</td>
<td>2 vials</td>
<td>4°C</td>
</tr>
<tr>
<td>10X Diluent M Concentrate</td>
<td>20 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Biotinylated Human HSP27 Antibody</td>
<td>1 vial</td>
<td>-20°C</td>
</tr>
<tr>
<td>100X Streptavidin-Peroxidase Conjugate (SP Conjugate)</td>
<td>80 µL</td>
<td>-20°C</td>
</tr>
<tr>
<td>Chromogen Substrate</td>
<td>8 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>12 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>20X Wash Buffer Concentrate</td>
<td>2 x 30 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Sealing Tapes</td>
<td>3</td>
<td>N/A</td>
</tr>
</tbody>
</table>
6. **MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 1 Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 1 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- 6 tubes to prepare standard or sample dilutions.

7. **LIMITATIONS**

- Do not mix or substitute reagents or materials from other kit lots or vendors.
8. **TECHNICAL HINTS**

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests.** A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
9. **REAGENT PREPARATION**

Equilibrate all reagents to room temperature (18-25°C) prior to use. Prepare fresh reagents immediately prior to use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

9.1  **1X Diluent M**

Dilute the 10X Diluent M Concentrate 1:10 with reagent grade water. Mix gently and thoroughly. *Store for up to 1 month at 4°C.*

9.2  **1X Wash Buffer**

Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water. Mix gently and thoroughly.

9.3  **1X Biotinylated HSP27 Detector Antibody**

9.3.1 The stock Biotinylated HSP27 Antibody must be diluted with 1X Diluent M according to the label concentration to prepare 1X Biotinylated HSP27 Antibody for use in the assay procedure. Observe the label for the “X” concentration on the vial of Biotinylated HSP27 Antibody.

9.3.2 Calculate the necessary amount of 1X Diluent M to dilute the Biotinylated HSP27 Antibody to prepare a 1X Biotinylated HSP27 Antibody solution for use in the assay procedure according to how many wells you wish to use and the following calculation:

<table>
<thead>
<tr>
<th>Number of Wells Strips</th>
<th>Number of Wells</th>
<th>( (V_T) ) Total Volume of 1X Biotinylated Antibody (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>32</td>
<td>1,760</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>2,640</td>
</tr>
<tr>
<td>8</td>
<td>64</td>
<td>3,520</td>
</tr>
<tr>
<td>10</td>
<td>80</td>
<td>4,400</td>
</tr>
<tr>
<td>12</td>
<td>96</td>
<td>5,280</td>
</tr>
</tbody>
</table>

*Any remaining solution should be frozen at -20°C.*
ASSAY PREPARATION

Where:

\( C_S \) = Starting concentration (X) of stock Biotinylated HSP27 Antibody (variable)

\( C_F \) = Final concentration (always = 1X) of 1X Biotinylated HSP27 Antibody solution for the assay procedure

\( V_T \) = Total required volume of 1X Biotinylated HSP27 Antibody solution for the assay procedure

\( V_A \) = Total volume of (X) stock Biotinylated HSP27 Antibody

\( V_D \) = Total volume of 1X Diluent M required to dilute (X) stock Biotinylated HSP27 Antibody to prepare 1X Biotinylated Antibody solution for assay procedures

Calculate the volume of (X) stock Biotinylated Antibody required for the given number of desired wells:

\[
\left( \frac{C_F}{C_S} \right) \times V_T = V_A
\]

Calculate the final volume of 1X Diluent M required to prepare the 1X Biotinylated HSP27 Antibody:

\[
V_T - V_A = V_D
\]

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your antibody vial for the actual concentration of antibody provided.

\( C_S = 50X \) Biotinylated HSP27 Antibody stock

\( C_F = 1X \) Biotinylated HSP27 Antibody solution for use in the assay procedure

\( V_T = 3,520 \mu L \) (8 well strips or 64 wells)

\[
(1X/50X) \times 3,520 \mu L = 70.4 \mu L
\]

3,520 \mu L - 70.4 \mu L = 3,449.6 \mu L

\( V_A = 70.4 \mu L \) total volume of (X) stock Biotinylated HSP27 Antibody required

\( V_D = 3,449.6 \mu L \) total volume of 1X Diluent M required to dilute the 50X stock Biotinylated Antibody to prepare 1X Biotinylated HSP27 Antibody solution for assay procedures
9.3.3 First spin the Biotinylated HSP27 Antibody vial to collect the contents at the bottom.

9.3.4 Add calculated amount $V_A$ of stock Biotinylated HSP27 Antibody to the calculated amount $V_D$ of 1X Diluent M. Mix gently and thoroughly.

9.4 **1X SP Conjugate**

Spin down the 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent M.

*Any remaining solution should be frozen at -20°C.*
10. STANDARD PREPARATIONS

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
- Any remaining standard should be stored at -20°C after reconstitution and used within 30 days.
- This procedure prepares sufficient standard dilutions for duplicate wells.

10.1 Reconstitution of the HSP27 Standard vial to prepare an 80 ng/mL HSP27 Standard #1.

10.1.1 First consult the HSP27 Standard vial to determine the mass of protein in the vial.

10.1.2 Calculate the appropriate volume of 1X Diluent M to add when resuspending the HSP27 Standard vial to produce a 80 ng/mL HSP27 Standard #1 by using the following equation:

\[
C_S = \text{Starting mass of HSP27 Standard (see vial label) (ng)}
\]
\[
C_F = 80 \text{ ng/mL HSP27 Standard #1 final required concentration}
\]
\[
V_D = \text{Required volume of 1X Diluent M for reconstitution (µL)}
\]

Calculate total required volume 1X Diluent M for resuspension:

\[
(C_S / C_F) \times 1,000 = V_D
\]

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

\[
C_S = 160 \text{ ng of HSP27 Standard in vial}
\]
\[
C_F = 80 \text{ ng/mL HSP27 Standard #1 final concentration}
\]
\[
V_D = \text{Required volume of 1X Diluent M for reconstitution}
\]

\[
(160 \text{ ng} / 80 \text{ ng/mL}) \times 1,000 = 2,000 \mu\text{L}
\]
10.1.3 First briefly spin the HSP27 Standard vial to collect the contents on the bottom of the tube.

10.1.4 Reconstitute the HSP27 Standard vial by adding the appropriate calculated amount $V_D$ of 1X Diluent M to the vial to generate the 80 ng/mL HSP27 **Standard #1**. Mix gently and thoroughly.

10.2 Allow the reconstituted 80 ng/mL HSP27 **Standard #1** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions.

10.3 Label five tubes #2 - 6.

10.4 Add 360 µL of 1X Diluent M to tube #2 - 6.

10.5 To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.

10.6 To prepare **Standard #3**, add 120 µL of the **Standard #2** into tube #3 and mix gently.

10.7 Using the table below as a guide, prepare subsequent serial dilutions.

10.8 1X Diluent M serves as the zero standard, 0 ng/mL (tube #6)
## Standard Dilution Preparation Table

<table>
<thead>
<tr>
<th>Standard #</th>
<th>Volume to Dilute (µL)</th>
<th>Step 10.1</th>
<th>Volume Diluent M (µL)</th>
<th>Total Volume (µL)</th>
<th>Starting Conc. (ng/mL)</th>
<th>Final Conc. (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>Step 10.1</td>
<td></td>
<td></td>
<td>80.00</td>
<td>20.00</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>80.00</td>
<td>360</td>
<td>480</td>
<td>20.00</td>
<td>5.000</td>
</tr>
<tr>
<td>3</td>
<td>120</td>
<td>20.00</td>
<td>360</td>
<td>480</td>
<td>1.250</td>
<td>0.313</td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>5.000</td>
<td>360</td>
<td>480</td>
<td>1.250</td>
<td>0.313</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>1.250</td>
<td>360</td>
<td>480</td>
<td>1.250</td>
<td>0.313</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>0</td>
<td>360</td>
<td>360</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

![Diagram showing the dilution process](image-url)
11. SAMPLE PREPARATION

11.1 Plasma
Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3,000 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).

11.2 Serum
Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 x g for 10 minutes. Remove serum and assay. Store serum at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.3 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 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.

11.4 Tissue
Extract tissue samples with 50 mM phosphate-buffered saline (pH 7.4) containing 1% Triton X-100 and centrifuge at 14,000 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.
11.5 Milk

Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. Milk dilution is suggested at 1:2 in 1X Diluent M. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well plate strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.
13. ASSAY PROCEDURE

- Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards and samples as instructed. Equilibrate reagents to room temperature before use. The assay is performed at room temperature (18-25°C).

13.2 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.

13.3 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.

13.4 Wash five times with 200 μL of 1X Wash Buffer manually. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 μL of 1X Wash Buffer and then invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid.

13.5 Add 50 μL of 1X Biotinylated HSP27 Antibody to each well and incubate for 2 hours.

13.6 Wash microplate as described above.

13.7 Add 50 μL of 1X SP Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.

13.8 Wash microplate as described above.

13.9 Add 50 μL of Chromogen Substrate per well and incubate for about 15 minutes or till the optimal blue colour density
develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.

13.10 Add 50 μL of Stop Solution to each well. The color will change from blue to yellow.

13.11 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 may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.
14. **CALCULATIONS**

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.
15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.
16. **TYPICAL SAMPLE VALUES**

**SENSITIVITY** –
The minimum detectable dose of HSP27 is typically 0.3 ng/mL.

**RECOVERY** –
Standard Added Value: 1.25 – 20 ng/mL
Recovery %: 89 – 112.
Average Recovery %: 97

**REFERENCE VALUE** –
The normal Human HSP27 plasma levels are <12 ng/mL.

**PRECISION** –

<table>
<thead>
<tr>
<th>% CV</th>
<th>Intra-Assay</th>
<th>Inter-Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.6</td>
<td>7.3</td>
</tr>
</tbody>
</table>

**LINEARITY OF DILUTION** –

<table>
<thead>
<tr>
<th>Plasma Dilution</th>
<th>Average % Expected Value Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Dilution</td>
<td>90</td>
</tr>
<tr>
<td>1:2</td>
<td>99</td>
</tr>
<tr>
<td>1:4</td>
<td>101</td>
</tr>
</tbody>
</table>
# 17. ASSAY SPECIFICITY

<table>
<thead>
<tr>
<th>Species</th>
<th>% Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canine</td>
<td>20</td>
</tr>
<tr>
<td>Monkey</td>
<td>50</td>
</tr>
<tr>
<td>Bovine</td>
<td>None</td>
</tr>
<tr>
<td>Mouse</td>
<td>None</td>
</tr>
<tr>
<td>Swine</td>
<td>50</td>
</tr>
<tr>
<td>Rat</td>
<td>20</td>
</tr>
<tr>
<td>Rabbit</td>
<td>None</td>
</tr>
</tbody>
</table>
## 18. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn't fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4°C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; concentrate samples</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td></td>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed wash wells as recommended</td>
</tr>
<tr>
<td></td>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
</tr>
<tr>
<td></td>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
</tr>
<tr>
<td></td>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaw cycles)</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>------------------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>Wells are insufficiently washed</td>
<td>Wash wells as per protocol</td>
<td>Wash wells as per protocol recommendations</td>
</tr>
<tr>
<td></td>
<td>washed</td>
<td>recommendations</td>
</tr>
<tr>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
<td></td>
</tr>
<tr>
<td>Waiting too long to read plate</td>
<td>Read plate immediately after</td>
<td>Read plate immediately after adding STOP solution</td>
</tr>
<tr>
<td>after adding STOP solution</td>
<td>adding STOP solution</td>
<td></td>
</tr>
<tr>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended.</td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
</tr>
<tr>
<td>Using incompatible sample type</td>
<td>Detection may be reduced or absent</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td>(e.g. Serum vs. cell extract)</td>
<td>in untested sample types</td>
<td></td>
</tr>
</tbody>
</table>