

HUMAN SOLUBLE HEAT SHOCK PROTEIN 70 (HSP70) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION
OF HUMAN sHSP70 CONCENTRATIONS IN
CELL LYSATES, SERUM AND EDTA PLASMA



FOR RESEARCH USE ONLY. NOT FOR USE
IN DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

ELISA NAME	HUMAN sHSP70 ELISA
Catalog No.	SK00712-01
Lot No.	
Formulation	96 T
Standard Range	156.25 -20000 pg/mL
Sensitivity	50 pg/mL
Sample Volume	100 µl
Sample Type	Cell Lysates, Serum, EDTA Plasma
Specificity	Human, Mouse, Rat sHSP70
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2-8°C

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INTRODUCTION

Human sHSP70 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human sHSP70 in cell lysates, serum and EDTA plasma. It contains recombinant human sHSP70 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human sHSP70. Results obtained with naturally occurring sHSP70 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human sHSP70.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human sHSP70 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any sHSP70 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for human sHSP70 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of sHSP70 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute reagents with those from other lots or sources.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors

have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Soluble HSP70 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human sHSP70.	712-01-01	1 plate
Soluble HSP70 Standard – 10 ng/vial of recombinant human sHSP70 in a buffered protein base with preservatives; lyophilized.	712-01-02	2 vials
Detection Antibody Concentrate – 105 µL/vial, 100-fold Concentrate of biotinylated antibody against human sHSP70 with preservatives; lyophilized.	712-01-03	1 vial
Positive Control – one vial of recombinant human sHSP70, lyophilized (optional)	712-01-04	1 vial
Streptavidin-HRP Conjugate - 60 µL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP with preservatives	SAHRP	1 vial
Dilution Buffer – 60 mL of buffered protein based solution with preservatives	DB01	1 bottle
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
TMB Substrate Solution - 11 mL of TMB substrate solution	TMB01	1 bottle
Stop Solution - 11 mL of 0.5M HCl	S-STOP	1 bottle
Plate Sealer	EAPS	1 piece
Plastic Pouch	P01	1 piece

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20°C or -70°C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted

Standard and Detection Antibody Concentrate Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 200-fold Concentrate and other components may be stored at 2 - 8°C for up to 6 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE COLLECTION AND STORAGE

Cell Lysates – Rinse cells two times with PBS, making sure to remove any remaining PBS after the second rinse. Solubilize cells at 1×10^7 cells/mL in Lysis Buffer* and allow samples to sit on ice for 15 minutes. Assay immediately or store at $\leq -70^\circ\text{C}$.

*1 mM EDTA, 0.5% Triton X-100, 10 $\mu\text{g}/\text{mL}$ Leupeptin, 10 $\mu\text{g}/\text{mL}$ Pepstatin, 100 μM PMSF, 3 $\mu\text{g}/\text{mL}$ Aprotinin in PBS, pH 7.2-7.4 (**not included in this kit**)

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay

immediately or aliquot and store samples at $\leq -20^\circ\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^\circ\text{C}$. Avoid repeated freeze-thaw cycles.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Cell Lysates – Before use, centrifuge samples at 2000 x g for 5 minutes and transfer the supernate to a clean tube. **Optimal dilutions should be determined by each laboratory for each application with a sample pretest.**

Serum and EDTA Plasma – no dilution is necessary, but should determine optimal dilution with a sample pretest to be sure.

Optimal dilutions should be determined by each laboratory for each application with a sample pretest.

Use polypropylene test tubes.

REAGENT PREPARATION

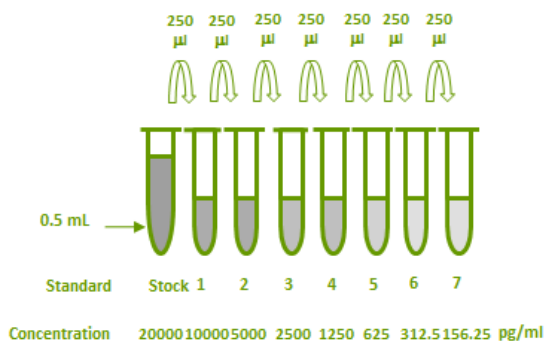
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

sHSP70 Standard - Refer to vial label for reconstitution volume. Reconstitute the sHSP70 standard with 0.5 mL of Dilution Buffer. This reconstitution produces a stock solution of 20,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 μL of the appropriate Dilution Buffer into tubes #1 to #7. Use the stock solution (20,000 pg/mL) to produce a dilution series (see below). Mix each tube thoroughly before the next transfer. The 20,000 pg/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 pg/mL). **Note: Use**

within one hour of reconstitution. A fresh standard should be used for each assay.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	500 µl	20000 pg/ml
# 1	250 µl of stock	250 µl	10000 pg/ml
# 2	250 µl of 1	250 µl	5000 pg/ml
# 3	250 µl of 2	250 µl	2500 pg/ml
# 4	250 µl of 3	250 µl	1250 pg/ml
# 5	250 µl of 4	250 µl	625 pg/ml
# 6	250 µl of 5	250 µl	312.5 pg/ml
# 7	250 µl of 6	250 µl	156.25 pg/ml



Detection Antibody - Reconstitute the Detection Antibody Concentrate with 105 µL of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of the appropriate Dilution Buffer into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution.

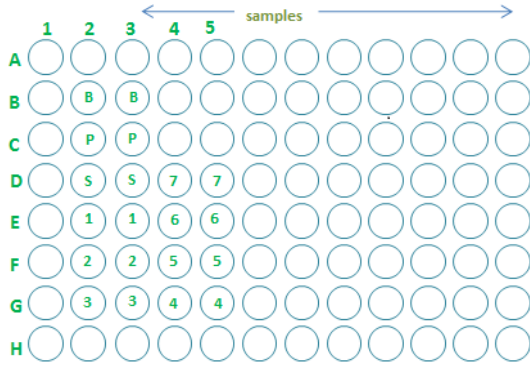
Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP conjugate should be used within a few days.

Positive Control (optional) - Reconstitute the positive control with 0.5 mL of Dilution Buffer to make positive control solution. **Note:** Positive Control should be used immediately.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
3. Add 100 µL of Dilution Buffer to Blank wells (B2, B3).
4. Add 100 µL of Standard (from D2, D3 to G2, G3 and D4, D5 to G4, G5), sample, or positive control (C2, C3) per well. Cover with the plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with the plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 1 hour on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 18-22 minutes at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and samples and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the sHSP70 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	O.D. CORRECTED (450NM)
Blank	0 (0.091)
78.125 (optional)	0.009
156.25	0.014
312.5	0.029
625	0.068
1250	0.129
2500	0.254
5000	0.428
10000	0.830
20000	1.602

- Lot No.:
- Positive Control: 1000 – 3000 pg/mL

CALIBRATION

This immunoassay is calibrated against a highly purified *E. Coli*-expressed recombinant human sHSP70.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of sHSP70 was 50 pg/mL.

SPECIFICITY

CYTOKINES	CROSS-REACTIVITY (%)
Human sHSP70	100
Mouse sHSP70	100
Rat sHSP70	100
Human HSP27	6.3
HSP60 (rh)	0
rhHSPA8	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin HRP conjugate working solution to each well. Incubate 1 hour on the plate shaker at RT. Protect from light.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 18-22 min on the plate shaker. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min