HUMAN SOLUBLE HEAT SHOCK PROTEIN 70 (HSP70) ELISA KIT

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

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

PRODUCT 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
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	Cell Lysates, Serum, EDTA Plasma
Specificity	Human, Mouse, Rat sHSP70
Calibration	Human sHSP70 Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 - 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human sHSP70 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human sHSP70 from cell lysates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human sHSP70 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural sHSP70 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human sHSP70. The capture antibody can bind to the human sHSP70 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human sHSP70 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human sHSP70 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix 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.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS 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 – 1.05 mL/vial, 10-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 concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. Streptavidin-HRP Conjugate 200-

fold concentrated solution 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. Microplate may be stored for up to 6 months at 2 - 8° C.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

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 x 10^7 cells/mL in Lysis Buffer* and allow samples to sit on ice for 15 minutes. Assay immediately or store at ≤ -70°C.

1 mM EDTA, 0.5% Triton X-100, 10 μg/mL Leupeptin, 10 μg/mL Pepstatin, 100 μM PMSF, 3 μg/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 ≤ -20° 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 ≤ -20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) 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.

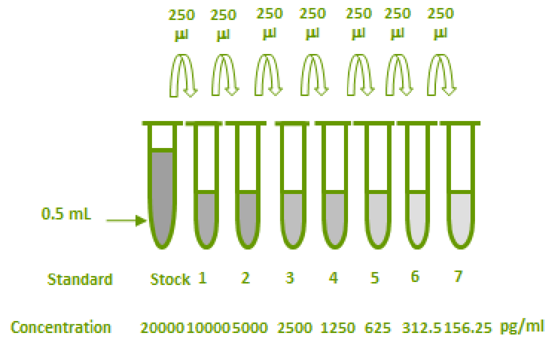
REAGENT PREPARATION

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 1x Wash Buffer.

sHSP70 Standard - 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 250μL 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



Positive Control (optional) - Reconstitute the positive control with 0.5 mL of Dilution Buffer to make positive control solution. **Note:** Positive Control could be reused within a few days if stored at -20 °C or -70 °C.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of the appropriate Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-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 (protect from light).

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

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.
3. Add 100 µL per well of Dilution Buffer to Blank wells.
4. Add 100 µL of Standard dilutions, sample, or positive control per well. Cover with the plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x 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 15-25 minutes on micro-plate shaker 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)	0.008
156.25	0.015
312.5	0.025
625	0.069
1250	0.125
2500	0.246
5000	0.403
10000	0.829
20000	1.548

- Lot No.:
- Positive Control:

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