Surveyor™ IC

Human/Mouse/Rat Total HSP70/HSPA1A Immunoassay

Catalog Number SUV1663

For the quantitative determination of Heat Shock Protein 70 (HSP70) in cell lysates.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Heat shock proteins (HSPs) are a family of highly conserved stress response proteins which function primarily as molecular chaperones that facilitate the folding of translated cellular proteins, prevent protein aggregation, and target improperly folded proteins to specific degradative pathways (1-3). While some heat shock proteins are constitutively expressed (e.g. HSP60 and HSP90β), many are expressed at low levels under normal physiological conditions but are dramatically upregulated in response to heat shock or other cellular stress (1-3). The HSP70 family of heat shock proteins consists of numerous HSP70 isoforms that are encoded by eleven distinct genes (4). HSP70-1A (also known as HSPA1A, HSP70-1, and HSP72) was the first HSP70 family member to be extensively characterized (4, 5). HSP70-1A is constitutively expressed at low levels but represents the major inducible form of HSP70 in response to heat shock or other cell stress stimuli (4, 5). All HSP70 family members share a 44 kDa ATPase domain that uses the hydrolysis of ATP to drive protein folding (6). In addition to its primary role as a molecular chaperone, a number of other functions have been reported for HSP70. It is a prominent cytoprotective factor in cells and elevated levels of HSP70 have been observed in association with ischemia/reperfusion, cancer, and chronic heart failure (7, 8). Cytoprotection by HSP70 may involve multiple mechanisms in addition to chaperoning denatured and aggregated proteins (9). For instance, there is considerable evidence that HSP70 functions as an anti-apoptotic molecule by down-regulating several apoptotic pathways. HSP70 has been shown to interfere with the assembly of the apoptosome, preventing the recruitment of pro-caspase-9 to APAF-1 and the subsequent activation of pro-caspase-3 (10, 11). HSP70 can also prevent apoptosis by blocking the release of apoptosis inducing factor (AIF) (12). Because of its central role in protecting cells from lethal stresses, HSP70 is a potential candidate as a therapeutic target for treatment of cancer and neurological diseases (8).

PRINCIPLE OF THE ASSAY

This Surveyor IC Immunoassay employs a two-site sandwich ELISA to quantitate HSP70 in cell lysates. An antibody specific for HSP70 has been pre-coated onto a microplate. Standards and samples are added and HSP70 present is bound by the immobilized antibody. After washing away unbound material, a biotinylated detection antibody recognizing HSP70 is used for detection utilizing a standard Streptavidin-HRP format. Substrate Solution is added to the wells and color develops in proportion to the amount of HSP70 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- This Surveyor IC Immunoassay 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.
- If samples generate values higher than the highest standard, further dilute the samples with Assay Diluent 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.
- Variations in sample collection, processing, and storage may cause sample value differences.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Total HSP70 Microplate	842238	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against HSP70.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Total HSP70 Standard	841682	2 vials (80 ng/vial) of recombinant human HSP70-1A in a buffered protein base with preservatives; lyophilized.	Use within 1 hour of reconstitution. Use a fresh standard for each assay.
Total HSP70 Detection Antibody	842239	3.75 µg of a biotinylated rabbit anti-HSP70 polyclonal antibody; lyophilized.	May be stored for up to 1 month at 2-8 °C.*
Sample Diluent Concentrate 1 (5X)	895562	2 vials (21 mL/vial) of a 5-fold concentrated buffer with preservatives.	
Reagent Diluent Concentrate 2 (10X)	841380	21 mL of a 10-fold concentrated solution of buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Streptavidin-HRP	890803	1.0 mL of streptavidin conjugated to horseradish-peroxidase.	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- 100 mL and 500 mL graduated cylinders.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Phosphate-buffered saline (PBS).
- Phenylmethylsulfonylfluoride (PMSF) (optional; Sigma, Catalog # P7626).
- Protease Inhibitor Cocktail (optional; Sigma, Catalog # P2714).
- **Polypropylene** test tubes for dilution of standards and samples.

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. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Assay Diluent - Add 20 mL of Sample Diluent Concentrate 1 (5X) to deionized or distilled water to prepare 100 mL of Assay Diluent. Prepare only enough diluent to run the assay.

Reagent Diluent 2 - Add 5 mL of Reagent Diluent Concentrate 2 (10X) to deionized or distilled water to prepare 50 mL of Reagent Diluent 2.

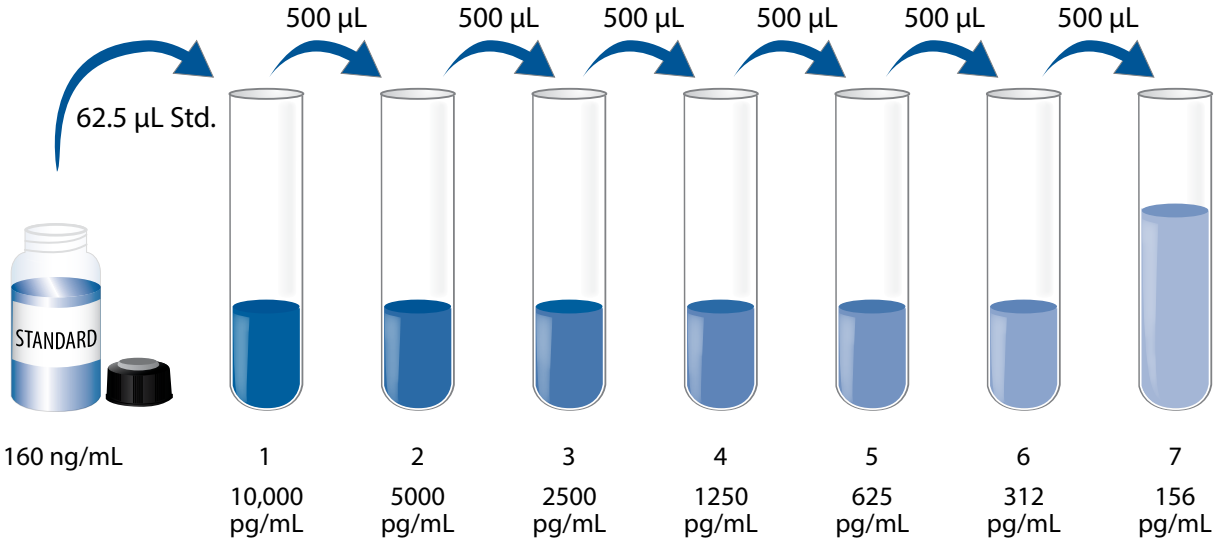
Total HSP70 Detection Antibody - Reconstitute the Total HSP70 Detection Antibody with 1.0 mL of Reagent Diluent 2. This reconstitution produces a stock solution of 3.75 µg/mL. Immediately before use, dilute the Detection Antibody to a working concentration of 0.25 µg/mL in Reagent Diluent 2.

Streptavidin-HRP - Immediately before use, dilute Streptavidin-HRP to the working concentration specified on the vial label using Reagent Diluent 2.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Prepare only enough Substrate Solution as needed to run the assay. Protect from light. 100 µL of the resultant mixture is required per well.

Total HSP70 Standard - Reconstitute the Total HSP70 Standard with 0.5 mL of Assay Diluent. This reconstitution produces a stock solution of 160 ng/mL. Mix the standard to ensure complete reconstitution. **Allow the standard to sit for a minimum of 15 minutes.**

Label seven **polypropylene tubes** 1 through 7. Add 937.5 µL of Assay Diluent into tube 1 and 500 µL of Assay Diluent into tubes 2 through 7. Add 62.5 µL of the 160 ng/mL Standard to tube 1. Mix thoroughly and continue to prepare a seven point standard curve using 2-fold serial dilutions by transferring 500 µL from tube 1 into tube 2 and subsequent 500 µL transfers as shown below. Use Assay Diluent as the zero standard. **Use a fresh standard for each assay. Use within 1 hour of preparation.**



CELL LYSIS PROCEDURE

Note: *It is recommended to supplement Assay Diluent with PMSF and the Protease Inhibitor Cocktail prior to use. Supplements should be used according to the manufacturer's instructions.*

1. Using PBS, collect non-adherent cells by centrifugation and adherent cells by scraping the culture flask.
2. Rinse cells two times with PBS, making sure to remove any remaining PBS after the second rinse.
3. Solubilize cells at 1 x 10^7 cells/mL in Assay Diluent.
4. Incubate on ice for 15 minutes. Centrifuge at 2000 x g for 5 minutes, and transfer the supernates into a clean test tube. Assay samples immediately or aliquot and store at ≤ -20 °C in a manual defrost freezer. Sample protein concentration may be quantified using a total protein assay.
5. If needed, further dilute samples in Assay Diluent.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Standard or sample per well. Use Assay Diluent as the zero standard. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
4. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (400 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.
5. Immediately before use, prepare the Total HSP70 Detection Antibody using Reagent Diluent 2. Add 100 µL of diluted Detection Antibody to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Immediately before use, prepare the Streptavidin-HRP. Add 100 µL of the diluted Streptavidin-HRP to each well. Incubate for 20 minutes at room temperature.
8. Repeat the aspiration/wash as in step 4.
9. Immediately before use, prepare the Substrate Solution. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. **Avoid placing the plate in direct light.**
10. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

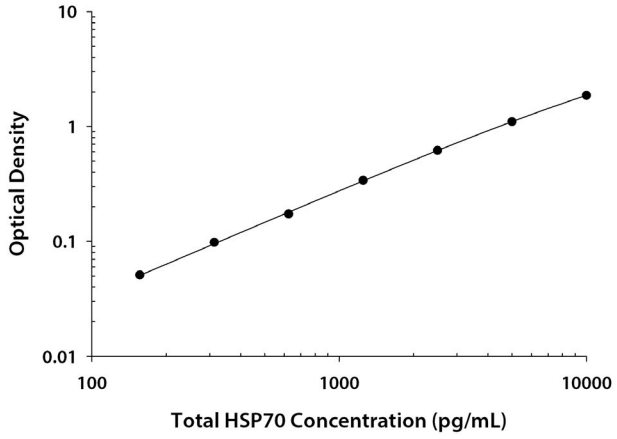
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Results may be normalized to total protein or cell number.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 HSP70 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.

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

TYPICAL DATA

A standard curve should be generated for each set of samples assayed. The graph below represents typical data generated when using this Human/Mouse/Rat Total HSP70/HSPA1A Surveyor IC Immunoassay. The standard curve was calculated using a computer generated 4-PL curve-fit. This standard curve is provided for demonstration purposes only.



(pg/mL)	O.D.	Corrected	Average
0	0.045	—	—
	0.053		
156	0.097	0.048	0.051
	0.102	0.053	
312	0.143	0.094	0.098
	0.150	0.101	
625	0.220	0.171	0.173
	0.223	0.174	
1250	0.386	0.337	0.339
	0.389	0.340	
2500	0.662	0.613	0.618
	0.672	0.623	
5000	1.139	1.090	1.100
	1.158	1.109	
10,000	1.887	1.838	1.864
	1.938	1.889	

CALIBRATION

The Human/Mouse/Rat Total HSP70/HSPA1A Surveyor IC Immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human HSP70-1A produced at R&D Systems. Samples containing natural HSP70 showed linear dilution parallel to the standard curve obtained using the Total HSP70 Standard. These results indicate that O.D. values from this Surveyor IC Immunoassay can be used to determine the concentration of HSP70 in natural samples.

SPECIFICITY

The Human/Mouse/Rat Total HSP70/HSPA1A Surveyor IC Immunoassay specifically recognizes HSP70. Specificity was demonstrated by Western blot analysis of the protein bound by the capture antibody coated on the plate.

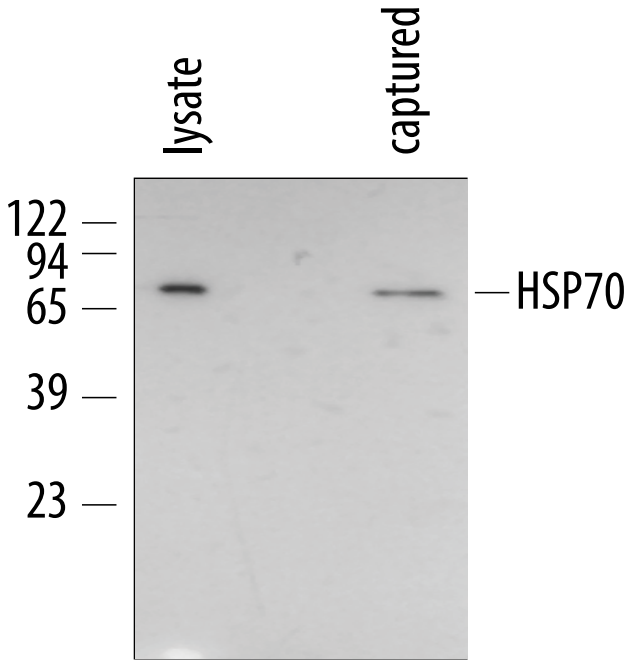


Figure 1: Lysates prepared from heat-shocked Jurkat human acute T cell leukemia cells were incubated in wells coated with the capture antibody. Unbound material was removed by washing and bound material was solubilized in SDS gel sample buffer. The same lysate and captured proteins were electrophoresed, transferred to a PVDF membrane, and immunoblotted with Total HSP70 Detection Antibody. Only a single band corresponding to HSP70 was detected.

To further determine specificity, recombinant human (rh) HSP27 was assayed at 100 ng/mL and measured 6294 pg/mL (6.3% cross-reactivity). Recombinant human HSP60, rhHSPA8, and rhGRP78/HSPA5 were assayed at 100 ng/mL and exhibited no cross-reactivity or interference in the assay.

QUANTIFICATION

Amounts of human HSP70, as measured by the Human/Mouse/Rat Total HSP70/HSPA1A Surveyor IC Immunoassay, are consistent with the relative amounts of HSP70 determined by qualitative Western blot analysis.

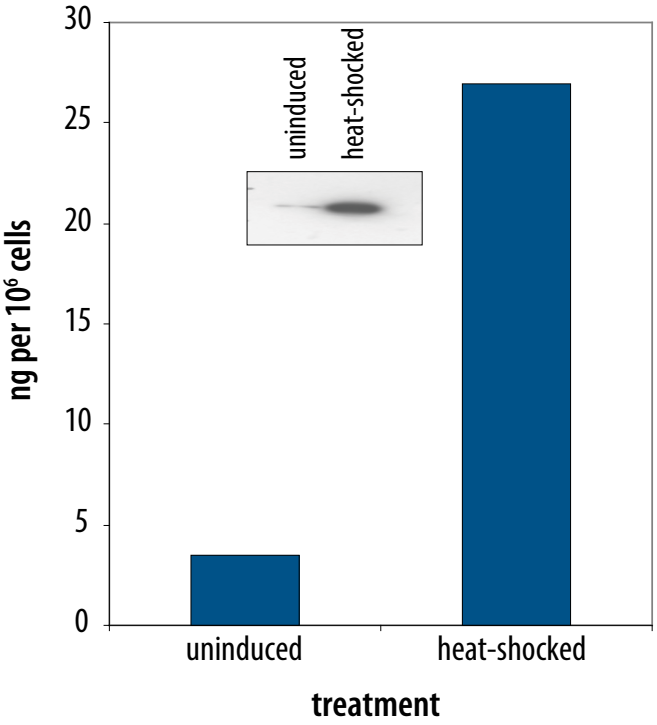


Figure 2: Jurkat human acute T cell leukemia cells were heat-shocked at 44 °C for 30 minutes and allowed to recover for 4 hours at 37 °C. Following cell lysis, HSP70 was quantified with this Surveyor IC Immunoassay. The same lysates were also immunoblotted (inset) with rabbit anti-HSP70 antibody (R&D Systems, Catalog # AF1663). The Surveyor IC Immunoassay results correlate well with the relative amounts of HSP70 detected by Western blot.

QUANTIFICATION *CONTINUED*

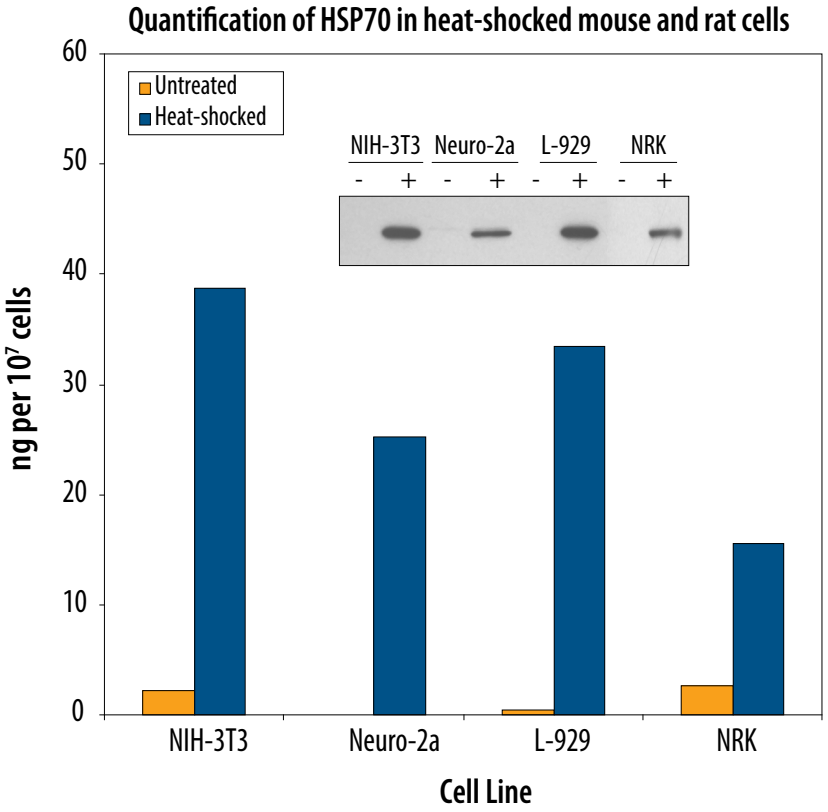


Figure 3: NIH-3T3 mouse embryonic fibroblast cells, Neuro-2a mouse neuroblastoma cells, L-929 mouse fibroblast cells, and NRK rat normal kidney cells were heat-shocked at 44 °C for 30 minutes and allowed to recover for 4 hours at 37 °C. Following cell lysis, HSP70 was quantified with this Surveyor IC Immunoassay. The same lysates were also immunoblotted (inset) with rabbit anti-HSP70 antibody (R&D Systems, Catalog # AF1663). The Surveyor IC Immunoassay results correlate well with the relative amounts of HSP70 detected by Western blot.

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