

ab133060 – HSP70 ELISA Kit

Instructions for Use

For quantitative detection and quantitation of HSP70 in cell lysates and tissue extracts.

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

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1. BACKGROUND

Abcam's HSP70 *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of HSP70 in samples from Human, Mouse and Rat origins. This assay allows for the quantitative determination of inducible HSP70 from cell lysates and tissue extracts.

A HSP70 monoclonal antibody has been precoated onto 96-well plates. Standards or test samples are added to the wells, incubated and then washed. A HSP70 polyclonal antibody is then added, incubated and washed. An HRP conjugated anti-IgG antibody is then added, incubated. The plate is washed once more and the TMB substrate is then added which HRP catalyzes, generating a blue coloration after incubation. A stop solution is added which generates conversion to yellow color read at 450 nm which is proportional to the amount of analyte bound.

The traditional method for HSP70 detection and quantitation is accomplished in two steps: immunoblotting followed by densitometry scanning. Assay Designs' HSP70 ELISA kit provides researchers with a rapid and reliable method to measure the levels of inducible HSP70 in numerous samples.

This kit has the potential of expanding our knowledge of HSP70's role in critical cellular processes or implicating HSP70 as a diagnostic tool to evaluate and monitor a variety of diseases.

Inducible heat shock protein 70 (Hsp70) is a stress protein whose expression is up-regulated when the cell or organism is placed under conditions of stress. Hsp70 is essential for cellular recovery, survival, and maintenance of normal cellular function. It is also a molecular chaperone that prevents protein aggregation and refolds damaged proteins in response to cellular stress caused by environmental insults, pathogens, and disease. Current research is aimed at exploiting

Hsp70's cellular protective abilities as a therapeutic strategy against damaging cellular stress.

In most mammals, the expression of inducible Hsp70 is strictly stress inducible and can only be detected following a significant stress upon the cell or organism. However, in humans and primates, inducible Hsp70 is present at basal levels and is up-regulated in response to stress. The role of Hsp70 has been studied in a variety of medically relevant models or conditions such as hyperthermia, hypertension, toxic exposure to chemical agents, hypoxia, ischemia, inflammation, autoimmunity, apoptosis, cancer, organ transplantation, and bacterial and viral infections. Hsp70 has also been studied in the normal processes of aging, spermatogenesis, menstruation, and physical activity such as exercise.

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 antibody to each well. Incubate at room temperature.

Label



Wash and add prepared Antibody-HRP Conjugate. Incubate at room temperature.

Substrate **Colored product**



Add TMB Substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

- Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up
- Stop Solution 2 is a 1 normal (1N) hydrochloric acid solution. This solution is caustic; care should be taken in use
- The activity of the Horseradish peroxidase conjugate is affected by nucleophiles such as azide, cyanide and hydroxylamine
- We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results

4. STORAGE AND STABILITY

Store kit at 4°C immediately upon receipt, apart from the Standard, which should be stored at -20°C. Avoid

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition
Anti-HSP70 Microplate (12 x 8 wells)	96 Wells	4°C
Anti-rabbit IgG HSP70 Horseradish Peroxidase Conjugate	10 mL	4°C
Recombinant HSP70 Standard	25 µL	-20°C
20X Wash Buffer Concentrate	100 mL	4°C
TMB Substrate	10 mL	4°C
Stop Solution 2	10 mL	4°C
Sample Diluent 2	50 mL	4°C
5X Extraction Reagent	10 mL	4°C
HSP70 Antibody	10 mL	4°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Standard microplate reader - capable of reading at 450 nm, preferably with correction between 570 and 590 nm
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes
- Microplate Shaker
- Absorbent paper for blotting
- Deionized water

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

8. TECHNICAL HINTS

- Standards can be made up in either glass or plastic tubes
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent
- Pipette standards and samples to the bottom of the wells
- Add the reagents to the side of the well to avoid contamination
- This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results
- It is important that the matrix for the standards and samples be as similar as possible. HSP70 samples diluted with Assay Buffer 13 should be run with a standard curve diluted in the same buffer. Serum samples should be evaluated against a standard curve run in Assay Buffer 13 while culture supernatant samples should be read against a standard curve diluted in the same complete but non-conditioned media
- A 5X Extraction Reagent has been included in this assay. Use of other lysis or extraction buffers may interfere with the performance of the assay
- **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 and samples to room temperature (18 - 25°C) prior to use.

9.1 **HSP70 Horseradish Peroxidase Conjugate**

Allow the HSP70 Horseradish Peroxidase Conjugate to equilibrate to room temperature. Any unused conjugate should be aliquoted and re-frozen at or below -20°C.

9.2 **1X Wash Buffer**

Prepare the 20X Wash Buffer by bringing to room temperature and swirl gently to dissolve any crystals that may have formed from storage Dilute 100 mL of the 20X Wash Buffer Concentrate in 1,900 mL of deionized water. Mix thoroughly and gently.

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Reconstitution of the HSP70 standard should be prepared no more than 1 hour prior to the experiment.

- 10.1 Centrifuge the Hsp70 standard before removing the cap.
- 10.2 Label eight tubes with numbers 1 – 8.
- 10.3 Add 500 μ L appropriate sample Diluent into tubes numbers 2 – 8.
- 10.4 Prepare a 50 ng/mL **Standard 1** by adding 5 μ L of the 10,000 ng/mL Stock Standard to 995 μ L of appropriate sample Diluent in to tube 1. Mix thoroughly and gently.
- 10.5 Prepare **Standard 2** by transferring 500 μ L from Standard 1 to tube 2. Mix thoroughly and gently.
- 10.6 Prepare **Standard 3** by transferring 500 μ L from Standard 2 to tube 3. Mix thoroughly and gently.
- 10.7 Using the table below as a guide, repeat for tubes 4 through 7.
- 10.8 **Standard 8** contains no protein and is the Blank control.

ASSAY PREPARATION

Standard	Sample to Dilute	Volume to Dilute (μL)	Volume of Diluent (μL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Stock	5	995	10,000	50
2	Standard 1	500	500	50	25
3	Standard 2	500	500	25	12.5
4	Standard 3	500	500	12.5	6.25
5	Standard 4	500	500	6.25	3.125
6	Standard 5	500	500	3.125	1.56
7	Standard 6	500	500	1.56	0.78
8	None	-	500	-	0



11. SAMPLE COLLECTION AND STORAGE

The HSP70 ELISA kit is compatible with HSP70 samples in a wide range of matrices after dilution in Assay Buffer. However, the end user must verify that the recommended dilutions are appropriate for their samples. Samples containing IgG may interfere with the assay.

11.1 SAMPLE PREPARATIONS

11.1.2 Extraction of samples

Please note that this assay is not appropriate for use with serum or plasma samples. Investigators may use alternative methods of cell and tissue lysate preparation, however, it is recommended that the 5X Extraction Reagent provided in this kit be diluted to 1X and used as the lysis buffer. Use of alternative lysis buffers may contain components which could interfere and compromise the performance of the assay, producing inaccurate results.

11.1.3 Dilution of samples

Sample Diluent 2 should be used to dilute lysates and tissue extracts and accompanying standards. Please note that this assay is not appropriate for use with serum or plasma samples.

A minimum 1:4 dilution of 1X Extraction Reagent into Sample Diluent 2 is required to remove matrix interference of this buffer. Due to differences in sample types, number of cells, or total cellular protein concentration, samples may require greater dilution with Sample Diluent 2 to remove interference or to be read within the range of the standard curve. Users must determine the optimal sample dilutions for their particular experiments.

Dilute prepared samples (i.e. cell and tissue lysates) in Sample Diluent 2. Prepare at least 250 μ L of diluted sample to permit assaying in duplicate. Mix thoroughly. Samples are now ready to

be used in the Assay Procedure. Samples must be kept on ice while reagents are being prepared.

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 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 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 directed in the previous sections.
 - 13.2 Add 100 μL of Standards 1 through 8 into the appropriate wells.
 - 13.3 Seal the plate and incubate for 2 hour at room temperature.
 - 13.4 Empty the contents of the wells and wash by adding 400 μL of 1X Wash Buffer to every well. Repeat the wash 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
 - 13.5 Add 100 μL of the HSP70 Antibody into each well.
 - 13.6 Cover wells with a fresh adhesive plate sealer or plastic wrap and incubate at room temperature for 1 hour, preferably with gentle mixing.
 - 13.7 Wash plate as described in step 13.4.
 - 13.8 Add 100 μL of HSP70 Conjugate to every well.
 - 13.9 Cover wells with a fresh adhesive plate sealer or plastic wrap and incubate at room temperature for 1 hour, preferably with gentle mixing.
 - 13.10 Wash plate as described in step 13.4.
 - 13.11 Add 100 μL of the TMB Substrate solution to every well. Incubate at room temperature for 30 minutes on a plate shaker.
 - 13.12 Add 100 μL Stop Solution 2 into each well in the same order that the TMB Substrate was added. The plate should be read immediately.

- 13.13 Read the O.D. absorbance at 450 nm, preferably with correction between 570 and 590 nm.

14. CALCULATIONS

A four parameter algorithm (4PL) provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

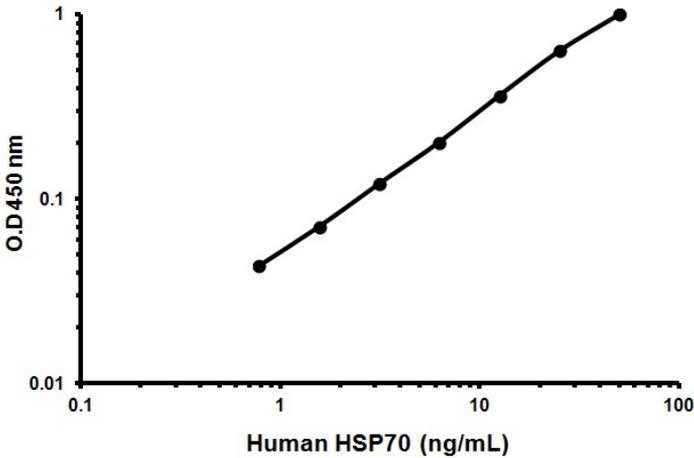
- Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average blank control OD from the average OD bound:

$$\text{Average Net OD} = \text{Average Bound OD} - \text{Average blank control OD}$$

- Plot the average Net OD for each standard versus HSP70 concentration in each standard. Sample concentrations may be calculated off of Net OD values using the desired curve fitting

15. TYPICAL DATA

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



Sample	Conc. (ng/mL)	Mean O.D. (-Blank)
Standard 1	50	1.000
Standard 2	25	0.635
Standard 3	12.5	0.363
Standard 4	6.25	0.203
Standard 5	3.125	0.121
Standard 6	1.56	0.071
Standard 7	0.78	0.043

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The sensitivity was calculated by first multiplying the concentration of the Low Standard (0.78 ng/mL) by two (2) standard deviations of the mean OD of twenty-four (24) replicates of B₀.

This value was then divided by the difference between the mean OD of twenty-four (24) replicates of the Low Standard and the mean OD of the twenty-four (24) replicates of B₀ Standard.

The sensitivity of the HSP70 ELISA has been determined to be 0.2 ng/mL. The standard curve has a range of 0.78 - 50 ng/mL.

LINEARITY OF DILUTION –

A buffer sample containing 30 ng/mL of recombinant HSP70 was serially diluted 1:2 in Sample Diluent 2 and measured in the assay. The results are shown in the table below.

Dilution	Expected (ng/mL)	Observed (pg/mL)	Recovery (%)
Neat	-	30.669	-
1:2	15.335	15.165	98.89
1:4	7.667	7.94	103.56
1:8	3.834	4.037	105.31
1:16	1.917	1.989	103.77
1:32	0.958	0.935	97.56

SAMPLE RECOVERY –

Recovery was determined by running serial dilutions of relevant samples (cell lysate and tissue extract) in Sample Diluent 2. The observed concentration of each sample was interpolated from the standard curve and then multiplied by the dilution performed to give the final sample concentration. Linearity was calculated at each dilution (excluding the last dilution).

Recoveries in which linearity fell between 85% and 115% were averaged to calculate the % recovery. The % recoveries for cell lysates and tissue extracts were > 90%. The percent linearity was calculated by dividing the final concentration of each dilution by the final concentration of the last dilution, whose observed concentration fell within the standard range. Linearity of the samples was achieved at or above 85%.

Below are examples of sample recoveries with several different types of samples. Note that % recovery was calculated based on linearity of samples.

Sample	Total Cellular Protein (mg/mL)	Recovery (%)	Recommended Dilution
Hela Cells	1.336	109	1:80
Heat-shocked HeLa Cells	1.296	106	1:80
Human Liver Microsomes	2.0	97	1:4

PRECISION –

Intra-Assay Precision (Within Run Precision)

To determine Intra-Assay Precision, 3 samples of known concentration were assayed 24 times on one plate. The Intra-Assay Coefficient of variation of Assay Designs' HSP70 ELISA has been determined to be < 5%.

Inter-Assay Precision (Between Run Precision)

To determine Inter-Assay Precision, 3 samples of known concentration were assayed 9 times in individual assays. The Inter-Assay Coefficient of variation of Assay Designs' HSP70 ELISA has been determined to be < 13%.

17. ASSAY SPECIFICITY

CROSS REACTIVITY –

Abcam's HSP70 ELISA kit is specific for both natural source and recombinant inducible HSP70.

The assay does not cross react with 5,000ng/mL of bovine constitutive Hsc70, recombinant hamster Grp78, E. coli DnaK, or recombinant M. tuberculosis Hsp71. The HSP70 ELISA has been certified for the detection of human, mouse, and rat inducible HSP70.

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed

19. NOTES

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