ab133061 – HSP70 High Sensitivity ELISA Kit

Instructions for Use

For quantitative detection and quantification of HSP70 in serum and plasma samples of Human, Mouse, and Rat origin

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

Version 2b Last Updated 2 January 2024

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1. BACKGROUND

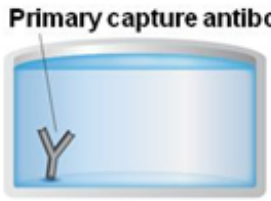
Abcam's HSP70 High Sensitivity *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of HSP70 in serum and plasma samples from human, mouse and rat origin. It does not detect other Hsp70 family members such as Hsc70 (Hsp73), Grp78, DnaK (*E. coli*), or Hsp71 (*M. tuberculosis*).

A HSP70 mouse 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.

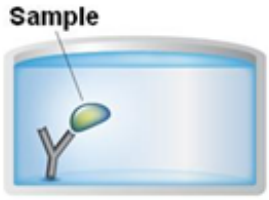
HSP70 is a molecular chaperone whose expression is induced upon exposure of the cell or organism to conditions of stress. It prevents protein aggregation and promotes the refolding of proteins that become damaged in response to environmental insults, pathogens, and disease. Its activity is essential for cellular survival and recovery under stress conditions, as well as for the maintenance of normal cellular function under non-stress conditions. HSP70 has been implicated to play a role in a variety of disease and physiological processes such as hyperthermia, hypertension, toxic exposure to chemical agents, hypoxia, ischemia, inflammation, autoimmunity, apoptosis, cancer, organ transplantation, and bacterial and viral infections. HSP70 is also a key regulator of many normal physiological processes including aging, spermatogenesis, menstruation, and physical activity such as exercise. The HSP70 High Sensitivity EIA kit is designed to evaluate and monitor HSP70 in these processes,

providing a key research tool to understand the role of HSP70 in physiology and disease.

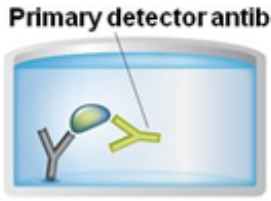
2. ASSAY SUMMARY



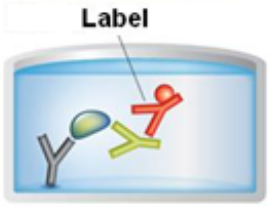
Prepare all reagents, samples and standards as instructed.



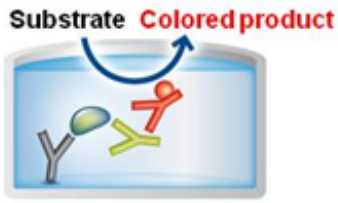
Add standard or sample to each well used. Incubate at room temperature.



Wash and add antibody to each well. Incubate at room temperature.



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



Add TMB 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.

- 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 multiple freeze-thaw cycles.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition
HSP70 High Sensitivity Microplate (12 x 8 wells)	96 Wells	4ºC
HSP70 Horseradish Peroxidase Conjugate	10 mL	4ºC
Recombinant HSP70 Standard	25 µL	-20ºC
Hsp70 Antibody	10 mL	4ºC
20X Wash Buffer Concentrate	100 mL	4ºC
TMB Substrate	10 mL	4ºC
Stop Solution 2	10 mL	4ºC
Assay Buffer 28	50 mL	4ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Deionized or distilled water
- Precision pipets for volumes between 5 µL and 1,000 µL
- Repeater pipet for dispensing 100 µL
- Disposable beakers for diluting buffer concentrates
- Graduated cylinders
- A microplate shaker
- Lint-free paper for blotting
- Microplate reader capable of reading at 450 nm
- Graph paper for plotting the standard curve

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 must be diluted in 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 28 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 28 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 High Sensitivity 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 High Sensitivity standard should be prepared no more than 1 hour prior to the experiment. Diluted standards should be used within 60 minutes of preparation if kept on ice; 20 minutes if kept at room temperature.

10.1 Allow the HSP70 High Sensitivity standard to equilibrate to room temperature.

10.2 Label eight tubes with numbers 1 – 8. Label another A.

10.3 Add 395 µL of assay buffer into tube A.

10.4 Add 900 µL assay buffer to tube 1 and 500 µL assay buffer to tubes 2 – 8.

10.5 Prepare **Intermediate Standard A** by adding 5 µL of the **Stock Standard** to tube A. Mix thoroughly and gently.

10.6 Prepare S**tandard** 1 by transferring 100 µL from tube A to 900 µL of the assay buffer to tube 1. Mix thoroughly and gently.

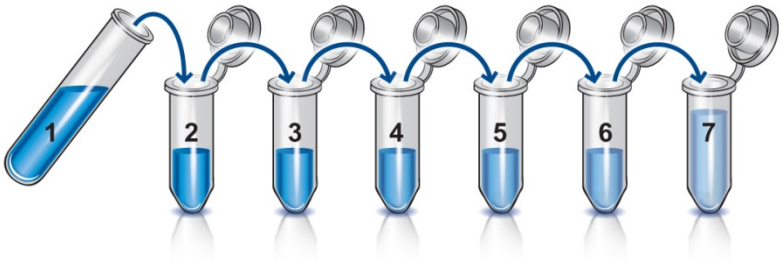
10.7 Prepare **Standard 2** by transferring 500 µL from Standard 1 to tube 2. Mix thoroughly and gently.

10.8 Prepare **Standard 3** by transferring 500 µL from Standard 2 to tube 3. Mix thoroughly and gently.

10.9 Using the table below as a guide, repeat for tubes 4 - 7.

10.10 **Standard 8** contains no protein and is the blank control.

Standard	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Intermediate Standard	100	900	125	12.50
2	Standard 1	500	500	12.50	6.25
3	Standard 2	500	500	6.25	3.13
4	Standard 3	500	500	3.13	1.56
5	Standard 4	500	500	1.56	0.78
6	Standard 5	500	500	0.78	0.39
7	Standard 6	500	500	0.39	0.20
8	None	-	500	-	0



11. SAMPLE COLLECTION AND STORAGE

11.1 This assay is suitable for measuring HSP70 in serum and EDTA plasma. Citrate and heparin plasma have not been validated for use. Prior to the assay, frozen samples should be slowly brought to 4˚C and centrifuged, if necessary, to remove residual debris. Hemolyzed and highly lipemic samples may interfere in the assay.

11.2 For serum and EDTA plasma, the minimal dilution required will vary for different samples. A 1:4 dilution into the assay buffer will remove matrix interference in the assay with most samples. However, due to variation in the samples, a different dilution may be required. Users must determine the optimal dilutions for their particular experiments.

11.3 Serum and Plasma Preparation

11.3.1 Collect whole blood in either clotting tubes for serum or EDTA tubes for plasma.

11.3.2 Allow serum to clot for 30 minutes.

11.3.3 Centrifuge at 1000 x g for 15 minutes at 4°C.

11.3.4 Place supernatants in a clean tube.

11.3.5 The supernatant may be aliquoted and stored at or below -20°C, or used immediately in the assay.

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 each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions
- 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

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 Add 100 µL of the Samples into the appropriate wells.

13.4 Seal the plate and incubate for 2 hours shaking at room temperature.

13.5 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.

13.6 Add 100 µL of the HSP70 High Sensitivity Antibody into every well.

13.7 Cover wells with a fresh adhesive plate sealer or plastic wrap and incubate at room temperature for 1 hour, preferably with gentle mixing.

13.8 Wash plate as described in step 13.5.

13.9 Add 100 µL of HSP70 High Sensitivity Conjugate to every well.

13.10 Cover wells with a fresh adhesive plate sealer or plastic wrap and incubate at room temperature for 1 hour, preferably with gentle mixing.

13.11 Wash plate as described in step 13.5.

13.12 Add 100 µL of the TMB Substrate solution to every well. Incubate at room temperature for 30 minutes on a plate shaker.

13.13 Add 100 µL Stop Solution into each well in the same order that the TMB Substrate was added. The plate should be read immediately.

13.14 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.

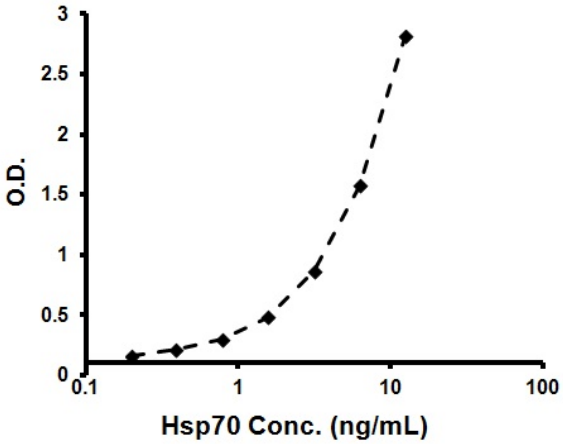
- Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average blank control OD from the average OD bound:

  Average Net OD = Average Bound OD - Average blank control OD

- Using linear graph paper, plot the Average Net OD for each standard versus HSP70 concentration in each standard. Approximate a straight line through the points. The concentration of HSP70 in the unknowns can be determined by interpolation

15. TYPICAL DATA

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



Sample	Net OD (-Blank)	Hsp70 ng/mL
Standard 1	2.810	12.50
Standard 2	1.574	6.25
Standard 3	0.864	3.13
Standard 4	0.481	1.56
Standard 5	0.299	0.78
Standard 6	0.211	0.39
Standard 7	0.163	0.20
Standard 8	0.104	0.00
Unknown1	1.291	4.98
Unknown 2	0.372	1.08

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The sensitivity or limit of detection of the assay is 0.09 ng/mL (90 pg/mL). The sensitivity was determined by interpolation at 2 standard deviations above the mean signal at background (0 ng/mL) using data from 7 standard curves.

LINEARITY OF DILUTION –

A buffer sample containing HSP70 was serially diluted 1:2 in Assay Buffer Diluent and measured in the assay. The results are shown in the table below.

Dilution	Expected (ng/mL)	Observed (ng/mL)	Recovery (%)
Neat	-	11.025	-
1:2	5.513	5.779	104.8
1:4	2.756	2.929	106.3
1:8	1.378	1.757	127.5
1:16	0.689	0.634	92.1
1:32	0.345	0.357	103.6
1:64	0.172	0.144	83.6

SAMPLE RECOVERY –

Below are examples of sample recoveries with several different types of samples. The samples were spiked at 3 different levels f the supplied standard and diluted 4-fold into the assay buffer. Percent recoveries were calculated as a ratio of observed to exp ected level.

Sample	Recovery (%)	Recommended Dilution
Serum (Human)	94.2	1:4
Plasma (Human)	79.3	1:4

PARALLELISM –

Samples diluted 1:4 - 1:8 show a parallel dose response to that of the recombinant standard.

PRECISION –

Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing HSP70 in a single assay.

	HSP70 High (ng/mL)	Intra-Assay %CV
Low	5.11	3.9
Medium	2.19	11.4
High	0.99	5.9

Inter-assay precision was determined by measuring buffer controls of varying HSP70 concentrations in multiple assays over several days.

	HSP70 High (ng/mL)	Inter-Assay %CV
Low	4.98	12.8
Medium	2.63	13.7
High	1.08	19.1

17. ASSAY SPECIFICITY

CROSS REACTIVITY –

The cross reactivities for a number of related compounds were determined by diluting cross reactants in the assay buffer at several concentrations. These samples were then measured in the assay.

Compound	Cross Reactivity (%)
Human HSP70	100
Rat HSP70	117.6
Human HSP70B'	5.4
Salmon HSP70	0.8
E.coli Dnak	0.5
Bovine HSC70	<0.016
Hamster GRP78	<0.016
M.tuberculosis HSP71	<0.016

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 all the components as directed.

19. NOTES

For all technical and commercial enquires please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)