

# **ab133063 – anti-HSP70 IgG/A/M Human ELISA Kit**

## Instructions for Use

For quantitative detection and quantification of anti-HSP70 IgG/A/M antibodies in serum samples from human origin.

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

# Table of Contents

---

## INTRODUCTION

- 1. BACKGROUND 2
- 2. ASSAY SUMMARY 5

## GENERAL INFORMATION

- 3. PRECAUTIONS 6
- 4. STORAGE AND STABILITY 7
- 5. MATERIALS SUPPLIED 7
- 6. MATERIALS REQUIRED, NOT SUPPLIED 8
- 7. LIMITATIONS 8
- 8. TECHNICAL HINTS 9

## ASSAY PREPARATION

- 9. REAGENT PREPARATION 10
- 10. STANDARD PREPARATIONS 11
- 11. SAMPLE COLLECTION AND STORAGE 13
- 12. PLATE PREPARATION 14

## ASSAY PROCEDURE

- 13. ASSAY PROCEDURE 15

## DATA ANALYSIS

- 14. CALCULATIONS 16
- 15. TYPICAL DATA 17
- 16. TYPICAL SAMPLE VALUES 18
- 17. ASSAY SPECIFICITY 19

## RESOURCES

- 18. TROUBLESHOOTING 20
- 19. NOTES 21

## 1. BACKGROUND

Abcam's anti-HSP70 IgG/A/M *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of anti-HSP70 antibodies in serum samples from human origin.

A recombinant Human Hsp70 protein has been precoated onto 96-well plates. Standards or test samples are added to the wells, incubated and then washed. An anti-Human GAM-HRP conjugated antibody is then added and 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.

Traditional methods for detection and quantitation anti-human HSP70 antibody were accomplished by using pre-screened serum samples with a high level of anti-human HSP70 antibody. These samples were assigned a concentration of 1000 arbitrary units/ml (Aunits/ml) and were used to generate standard dose-response curves from which antibody levels in test samples were determined.

The Anti-Human HSP70 (IgG/A/M) ELISA kit uses a calibrated standard of anti-human HSP70 (IgG/A/M) antibodies isolated from pooled human sera to generate a standard curve. The kit provides researchers with a rapid, reliable and standardized method to measure the levels of antihuman HSP70 antibody levels in human serum samples by interpolating absorbance readings from the standard curve. This kit has the potential of expanding our knowledge of the role of anti-human HSP70 antibodies in the normal population and as a diagnostic tool to evaluate and monitor a variety of diseases.

The inducible heat shock protein, HSP70 (Hsp72) is part of the HSP70 family which contains a number of highly related protein isoforms

ranging in size from 66 kDa to 78 kDa. These proteins include both cognate members which are found within major intracellular compartments and highly inducible isoforms which appear to be predominantly cytoplasmic or nuclear in distribution. Members of the HSP70 family are molecular chaperones that are involved in many cellular functions such as protein folding, transport, maturation and degradation, exerting their function in an ATP-dependent manner. The molecular chaperones of the HSP70 family recognize and bind to nascent polypeptide chains as well as partially folded intermediates of proteins preventing their aggregation and misfolding. Inducible

HSP70 is typically regarded as an intracellular protein. However studies have shown the presence of soluble HSP70 and anti-HSP70 antibodies in the peripheral circulation of normal individuals and in various disease states in the following instances.

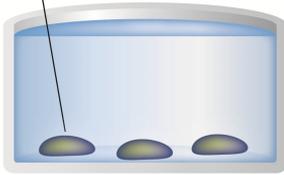
The presence of circulating anti-HSP70 antibodies was detected more frequently in smokers versus non-smokers. In patients with Graves' disease higher anti-HSP70 antibody levels were measured compared to controls. Patients with uveitis were found to have circulating levels of anti-HSP70 antibody and these levels may reflect the extent of disease involvement within the eye. Antibodies against various heat shock proteins including HSP70 were detected in sera of patients with dilated cardiomyopathy as compared to healthy controls. A correlation between anti-HSP70 antibodies and different types of vascular diseases exists suggesting that HSP70 might be involved in the pathogenesis and propagation of arteriosclerosis. There is a possible association of plasma anti-HSP70 antibody levels with hypertension and harsh working conditions. Patients with severe heat-induced symptoms showed significantly higher anti-HSP70 antibody levels. Antibodies to HSP70 have been associated with graft-versus-host disease in peripheral blood stem cell transplant recipients. HSP70 has been implicated as a potential auto antigen in multiple sclerosis and enhanced expression of several heat shock proteins including HSP70 in myelin may subsequently present as additional immune targets involved in the progression of this disease. Anti-HSP70 antibodies may

be involved in the pathogenesis of schizophrenia and especially high anti-HSP70 titers were found in never-medicated patients. Formation of HSP70-antibody complexes in the placenta correlated with anti-HSP70 antibody levels in sera and these complexes may contribute to the induction of preterm birth. Women sensitized to these antibodies may be at increased risk for adverse pregnancy outcomes. HSP70 and anti-HSP70 antibodies may have diagnostic and prognostic value for different gynaecologic malignancies.

The ubiquitous nature of HSP70 and the high degree of sequence homology between mammals and bacterial heat shock proteins may provide a link between infection and autoimmunity. Further studies are required to evaluate the physiological and immunological relevance of circulating HSP70 and anti-HSP70 antibodies and their interaction in autoimmune and inflammatory conditions.

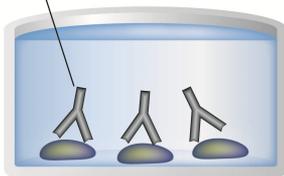
## 2. ASSAY SUMMARY

Capture Antigens



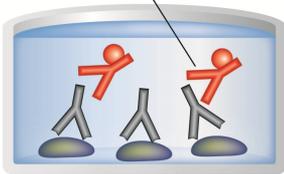
Prepare all reagents, samples and standards as instructed.

Sample



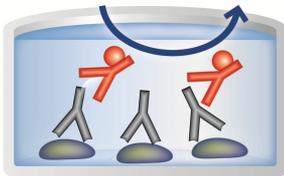
Add samples and standards to wells and incubate.

Labeled HRP-Conjugate



Add prepared labeled HRP-Conjugate. Incubate at room temperature.

Substrate      Colored Product



After washing, add TMB substrate solution 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
- The standard is derived from human serum. Treat as a biohazard

## 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 (Before Preparation)
Anti-HSP70 Microplate (12 x 8 wells)	96 Wells	4°C
Anti-Human HSP70-HRP Conjugate	10 mL	4°C
Sample Diluent 2	100 mL	4°C
Anti-Human HSP70 Standard	120 µL	-20°C
20X Wash Buffer Concentrate	100 mL	4°C
TMB Substrate	10 mL	4°C
Stop Solution 2	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:

- Deionized or distilled water
- Precision pipettes capable of accurately delivering 1 to 1,000  $\mu\text{L}$
- Disposable pipette tips
- 5, 10, 25mL pipettes for reagent preparation
- 1L Graduated cylinder
- Squirt bottle, manifold dispenser, or automated microtiter plate washer
- Disposable polypropylene tubes
- Microtiter plate reader capable of measuring absorbance at 450nm

### **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 should be made up in disposable 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
- **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 **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. This can be stored at room temperature until the kit's expiration date, or for 4 weeks whichever comes first.

*NOTE:* 100mL of 20X Wash Buffer has been provided in this kit, which is sufficient for the preparation of 2L of 1X Wash Buffer. The minimum required volume of 1X Wash Buffer is 310 mL (if the complete plate is used at once). However additional 1X Wash Buffer is supplied to allow for multiple assays or alternative washing techniques.

## 10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Diluted standards should be used within 70 minutes of preparation.

- 10.1 Allow the reconstituted 10,000 ng/mL anti-HSP70 IgG/A/M **Stock Standard** solution to warm to room temperature. The standard solution should be stored at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.
- 10.2 Label seven tubes with numbers 1 – 7.
- 10.3 Add 500 µL sample diluent to tubes 2-7.
- 10.4 Prepare a 1,000 ng/mL **Standard 1** by adding 100 µL of the 1,000 ng/mL Stock Standard to 900 µL of the sample diluent to tube 1. Mix thoroughly and gently.
- 10.5 Prepare **Standard 2** by transferring 500 µL from Standard 1 to tube 2. Mix thoroughly and gently.
- 10.6 Prepare **Standard 3** by transferring 500 µL from Standard 2 to tube 3. Mix thoroughly and gently.
- 10.7 Using the table below as a guide, repeat for tubes 4 through 6.
- 10.8 **Standard 7** contains no protein and is the blank control.

# ASSAY PREPARATION

Standard	Sample to Dilute	Volume to Dilute ( $\mu\text{L}$ )	Volume of Diluent ( $\mu\text{L}$ )	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Stock	100	900	10,000	1,000
2	Standard 1	500	500	1,000	500
3	Standard 2	500	500	500	250
4	Standard 3	500	500	250	125
5	Standard 4	500	500	125	62.5
6	Standard 5	500	500	62.5	31.25
7	None	-	500	-	0



## **11. SAMPLE COLLECTION AND STORAGE**

### **11.1 Collection of serum -**

- 11.1.1 Collect whole blood using a serum separator tube.
- 11.1.2 Allow samples to clot at room temperature for 30 minutes.
- 11.1.3 Centrifuge at approximately 1000 x g for 10 minutes, taking precautions to avoid hemolysis.
- 11.1.4 Remove serum. Transfer the serum to a labeled polypropylene tube. The serum collected is now ready for analysis using Assay Desins' Anti-Human HSP70 (total) ELISA Kit.
- 11.1.5 Alternatively, the serum sample can be frozen at  $\leq -20^{\circ}\text{C}$  and assayed at a later date. It is recommended that the serum be aliquoted to convenient volumes prior to storing at  $\leq -20^{\circ}\text{C}$  to avoid multiple freeze thaw cycles.

### **11.2 Dilution of samples -**

Serum can be diluted 1:1000 in Sample Diluent 2 by a 2 step serial dilution (1:10 dilution followed by a 1:100 dilution). This is a suggested starting dilution only. Additional dilutions may be necessary to ensure that sample values are within the range of the standard curve. Users must determine the optimal sample dilutions for their particular experiments.

- 11.2.1 Dilute prepared samples in Sample Diluent 2. Prepare at least 250  $\mu\text{L}$  of diluted sample to permit assaying in duplicate.
- 11.2.2 Mix thoroughly.
- 11.2.3 Samples are now ready to be used in the Assay Procedure. Samples may be left at room temperature 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 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  $\mu$ L of Standards 1 to 6 into the appropriate wells.
  - 13.3 Add 100  $\mu$ L 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  $\mu$ L 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  $\mu$ L of anti-Human Ig-HRP conjugate to 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  $\mu$ L of the TMB Substrate solution to every well. Incubate at room temperature for 15 minutes on a shaker.
  - 13.10 Add 100  $\mu$ L Stop Solution 2 into each well in the same order that the TMB Substrate was added. The plate should be read immediately.
  - 13.11 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.

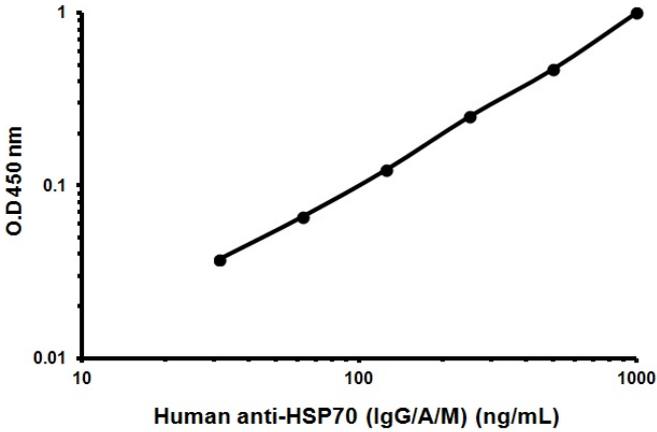
- Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average blank control OD from the average OD bound:

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

- Using linear graph paper, plot the Average Net OD for each standard versus anti-HSP70 IgG/A/M concentration in each standard. Approximate a straight line through the points. The concentration of anti-HSP70 IgG/A/M 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	Conc. (ng/mL)	Mean O.D. (-Blank)
Standard 1	1,000	1.000
Standard 2	500	0.471
Standard 3	250	0.251
Standard 4	125	0.123
Standard 5	62.5	0.066
Standard 6	31.25	0.037

## **16. TYPICAL SAMPLE VALUES**

### **SENSITIVITY –**

The sensitivity of the Anti-Human HSP70 (total) ELISA was determined to be 6.79ng/mL.

### **PRECISION –**

#### **Intra-Assay Precision (Within Run Precision)**

To determine Intra-Assay Precision, samples containing low, medium and high concentrations of anti-human HSP70 were assayed sixteen times on one plate. The Intra-Assay coefficient of variation of the Anti-Human HSP70 (total) ELISA was determined to be <10%.

#### **Inter-Assay Precision (Between Run Precision)**

To determine Inter-Assay Precision, three samples containing low, medium and high concentrations of anti-human HSP70 were assayed in eight individual assays. The Inter-Assay coefficient of variation of the Anti-Human HSP70 (total) ELISA was determined to be <10%.

### **LINEARITY –**

To determine linearity, a sample containing 792.0 ng/mL of antihuman HSP70 was diluted 1:2 in Sample Diluent 2 four times and measured in the assay. The data was plotted graphically as actual anti-human HSP70 concentration versus measured anti-human HSP70 concentration. The line obtained had a slope of 1.071 and a correlation coefficient of 0.9998.

## **17. ASSAY SPECIFICITY**

### **CROSS REACTIVITY –**

The anti-HSP70 IgG/A/M EIA detects Human anti-HSP70 IgG/A/M.  
The cross reactivity with other species as not been tested.

## 18. TROUBLESHOOTING

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
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



**UK, EU and ROW**

Email: [technical@abcam.com](mailto:technical@abcam.com) | Tel: +44-(0)1223-696000

**Austria**

Email: [wissenschaftlicherdienst@abcam.com](mailto:wissenschaftlicherdienst@abcam.com) | Tel: 019-288-259

**France**

Email: [supportscientifique@abcam.com](mailto:supportscientifique@abcam.com) | Tel: 01-46-94-62-96

**Germany**

Email: [wissenschaftlicherdienst@abcam.com](mailto:wissenschaftlicherdienst@abcam.com) | Tel: 030-896-779-154

**Spain**

Email: [soportecientifico@abcam.com](mailto:soportecientifico@abcam.com) | Tel: 911-146-554

**Switzerland**

Email: [technical@abcam.com](mailto:technical@abcam.com)

Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

**US and Latin America**

Email: [us.technical@abcam.com](mailto:us.technical@abcam.com) | Tel: 888-77-ABCAM (22226)

**Canada**

Email: [ca.technical@abcam.com](mailto:ca.technical@abcam.com) | Tel: 877-749-8807

**China and Asia Pacific**

Email: [hk.technical@abcam.com](mailto:hk.technical@abcam.com) | Tel: 400 921 0189 / +86 21 2070 0500

**Japan**

Email: [technical@abcam.co.jp](mailto:technical@abcam.co.jp) | Tel: +81-(0)3-6231-0940

[www.abcam.com](http://www.abcam.com) | [www.abcam.cn](http://www.abcam.cn) | [www.abcam.co.jp](http://www.abcam.co.jp)