ab133059 – Anti-HSP60 IgG/A/M Human ELISA Kit

Instructions for Use

For quantitative detection of Human Anti-HSP60 IgG/A/M in Human serum samples.

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

Version 3 Last Updated 5 July 2018

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1. BACKGROUND

Abcam's Human Anti-HSP60 IgG/A/M *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Human Anti-HSP60 in Human Serum. This assay allows determination of IgG, IgA and IgM antibodies (total) to Human Hsp60 in serum.

A recombinant Human Hsp60 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 of antihuman Hsp60 antibodies were accomplished by using prescreened serum samples with a high level of anti-Human Hsp60. 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.

Assay Designs Anti-Human Hsp60 (total) ELISA kit uses a calibrated standard of anti-Human Hsp60 (lgG/A/M) 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 anti-Human Hsp60 in Human serum samples by interpolating absorbance readings from the standard curve.

2. ASSAY SUMMARY

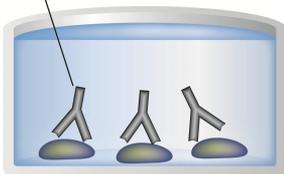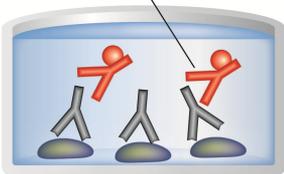**Capture Antigens**
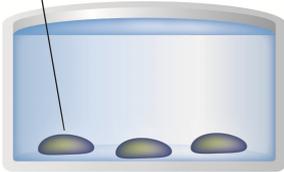
Prepare all reagents, samples and standards as instructed.

Sample

Add samples and standards to wells.

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. <u>PRECAUTIONS</u>

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 anti-Human GAM-HRP 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 anti-HSP60 standard is derived from Human serum. Treat as a biohazard**

4. STORAGE AND STABILITY

Store all components at 4°C immediately upon receipt. Avoid multiple freeze-thaw cycles.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition
Human Anti-HSP60 IgG/A/M Microplate (12 x 8 wells)	96 Wells	4ºC
Anti-Human GAM-HRP Conjugate	10 mL	4ºC
Human Anti-HSP60 Standard	120 µL	4ºC
20X Wash Buffer Concentrate	100 mL	4ºC
TMB Substrate	10 mL	4ºC
Stop Solution 2	10 mL	4ºC
Sample Diluent 1	100 mL	4ºC

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. <u>TECHNICAL HINTS</u>

- 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. Human Anti-HSP60 IgG/A/M samples diluted with Sample Diluent 1 should be run with a standard curve diluted in the same buffer. Serum samples should be evaluated against a standard curve run in Sample Diluent 1 while culture supernatant samples should be read against a standard curve diluted in the same complete but non-conditioned media

- **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 equilibrating 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. Dilutions of the Anti-HSP60 IgG/A/M standard should be used within 60 minutes of preparation. **The anti-HSP60 standard is derived from Human serum. Treat as a biohazard**.

10.1 Allow the anti-HSP60 standard to equilibrate to room temperature. Centrifuge the anti-HSP60 standard before removing cap.

10.2 Label seven tubes with numbers 1 – 7.

10.3 Add 500 μL appropriate diluent into tubes numbers 2 – 7.

10.4 Prepare a 250 ng/mL **Standard 1** by transferring 100 μL of the 2,500 ng/mL Stock Standard to 900 μL of the appropriate 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.

Standard	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Stock	100	900	2,500	250
2	Standard 1	500	500	250	125
3	Standard 2	500	500	125	62.5
4	Standard 3	500	500	62.5	31.25
5	Standard 4	500	500	31.25	15.63
6	Standard 5	500	500	15.63	7.81
7	None	-	500	-	0

11. SAMPLE COLLECTION AND STORAGE

- The Anti-HSP60 IgG/A/M Human ELISA kit is compatible with Anti-HSP60 IgG/A/M 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 Human IgG may interfere with the assay.

SAMPLE PREPARATIONS

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 1,000 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 the Anti-Human Hsp60 (total) ELISA Kit.

11.1.5 Alternatively, the serum sample can be frozen at ≤-20°C and assayed at a later date. It is recommended that the serum be aliquoted to convenient volumes prior to storing at ≤-20°C to avoid multiple freeze thaw cycles.

11.2 Dilution of samples

11.2.1 Serum can be diluted 1:1000 (v/v) in Sample Diluent 1 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.

11.2.2 Dilute prepared samples in Sample Diluent 1. Prepare at least 250 µL of diluted sample to permit assaying in duplicate.

11.2.3 Mix thoroughly.

11.2.4 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. 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 7 into the appropriate wells.

    13.3 Add 100 µL of samples into the appropriate wells.

    13.4 Incubate the plate at room temperature for 2 hours.

    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 Anti-Human GAM-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 the plates with 1X Wash Buffer, as detailed in Step 13.5.

    13.9 Add 100 µL of the TMB Substrate solution to every well. Incubate at room temperature for 15 minutes on a plate shaker.

    13.10 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.

    13.11 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.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.
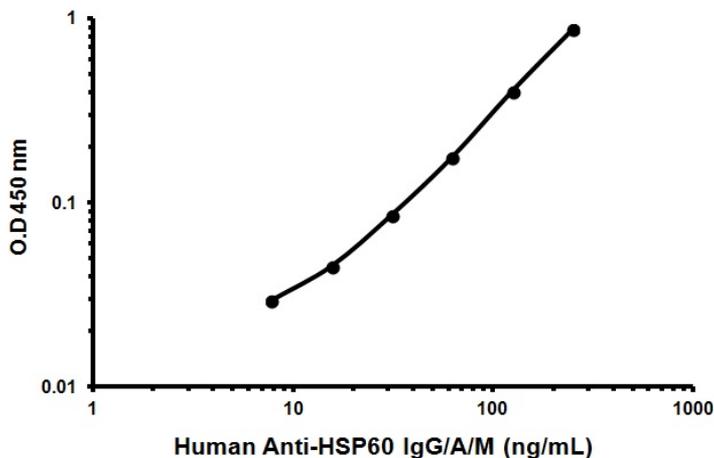
Sample	Conc. (ng/mL)	Mean O.D. (-Blank)
Standard 1	250	0.873
Standard 2	125	0.405
Standard 3	62.5	0.176
Standard 4	31.25	0.086
Standard 5	15.63	0.045
Standard 6	7.81	0.029

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The sensitivity, minimum detectable dose of Anti-HSP60 IgG/A/M using this Abcam ELISA kit was found to be 2.88 ng/mL. This was determined by the average optical density of the 0 pg/mL Standard and comparing to the average optical density for Standard 6. The detection limit was determined as the concentration of Anti-HSP60 IgG/A/M measured at two standard deviations from the zero along the standard curve.

LINEARITY OF DILUTION –

A sample containing 189.3 ng/mL Anti-HSP60 IgG/A/M was diluted 5 times 1:2 in the kit Sample Diluent 1 and measured in the assay. The data was plotted graphically as actual Anti-HSP60 IgG/A/M concentration versus measured Anti-HSP60 IgG/A/M concentration.

The line obtained had a slope of 0.948 and a correlation coefficient of 0.998.

PRECISION –

Intra-Assay Precision

To determine Intra-Assay Precision, samples containing low, medium and high concentrations of anti-Human Hsp60 were assayed sixteen times on one plate. The Intra-Assay coefficient of variation of the Anti-Human Hsp60 (total) ELISA was determined to be <10%.

b) Inter-Assay Precision

To determine Inter-Assay Precision, three samples containing low, medium and high concentrations of anti-Human Hsp60 were assayed in eight individual assays. The Inter-Assay coefficient of variation of the Anti-Human Hsp60 (total) ELISA was determined to be <10%.

17. ASSAY SPECIFICITY

CROSS REACTIVITY –

The Anti-HSP60 IgG/A/M ELISA Kit recognizes natural and recombinant forms of Anti-HSP60 IgG/A/M.

There was no Cross reactivities with the Anti-HSP60 IgG/A/M ELISA kit.

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. <u>NOTES</u>

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UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 108008523689 (中國聯通)

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940
www.abcam.com | www.abcam.cn | www.abcam.co.jp