Product Manual

HSP70 High Sensitivity ELISA kit

Catalog #: ADI-EKS-715

96 Well Kit

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Product Manual

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

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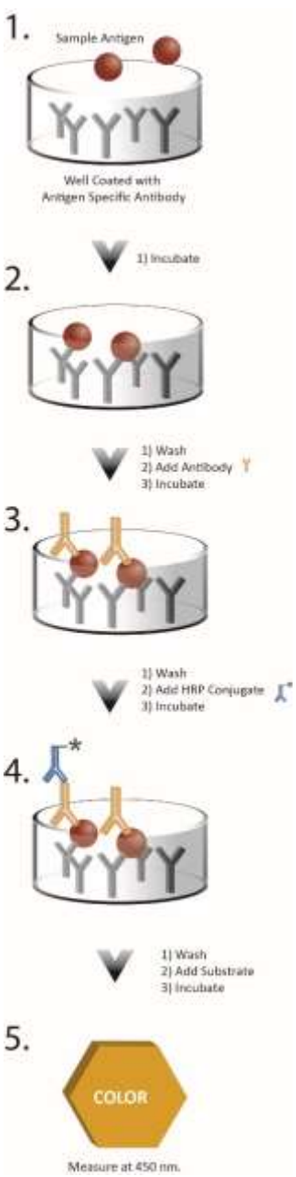


INTRODUCTION

The HSP70 high sensitivity ELISA kit is a complete kit for the quantitative determination of inducible Heat Shock Protein 70 (Hsp70) in serum and plasma samples of 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*). Please read the entire kit insert before performing this assay.

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[1-3]. Hsp70 has been implicated to play a role in a variety of disease and physiological processes such as hyperthermia,[4] hypertension,[5] toxic exposure to chemical agents,[6] hypoxia,[7] ischemia,[8,9] inflammation,[10] autoimmunity,[5, 11] apoptosis,[12, 13] cancer,[13] organ transplantation,[14] and bacterial[15,16] and viral[17] infections. Hsp70 is also a key regulator of many normal physiological processes including aging,[12,18] spermatogenesis,[19,20] menstruation,[21] and physical activity such as exercise[22]. The Hsp70 High Sensitivity ELISA 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.

PRINCIPLE

1. Samples and standards are added to wells coated with a monoclonal antibody specific for Hsp70. The plate is then incubated.
2. The plate is washed, leaving only bound Hsp70 on the plate. A yellow solution of polyclonal antibody, specific for Hsp70, is then added. This binds the Hsp70 captured on the plate. The plate is then incubated.
3. The plate is washed to remove excess antibody. A blue solution of HRP conjugate is added to each well, binding to the antibody. The plate is again incubated.
4. The plate is washed to remove excess HRP conjugate. TMB Substrate solution is added. An HRP-catalyzed reaction generates blue color in the solution.
5. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450nm. The amount of signal is directly proportional to the level of Hsp70 in the sample.

MATERIALS SUPPLIED

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

1. Assay Buffer 28

   50 mL, Catalog No. 80-1599

   Phosphate buffered saline containing BSA and detergent

2. Hsp70 High Sensitivity Standard

   25 µL, Catalog No. 80-1776

   One vial containing 10 µg/mL of recombinant human Hsp70

The standard should be handled with care due to the known and unknown effects of the molecule.

3. Hsp70 Clear Microtiter Plate

   One Plate of 96 Wells, Catalog No. 80-1581

   A plate of break-apart strips coated with a mouse monoclonal antibody specific for Hsp70

4. Wash Buffer Concentrate

   100 mL, Catalog No. 80-1287

   Tris buffered saline containing detergents

Activity of conjugate is affected by nucleophiles such as azide, cyanide, and hydroxylamine.

5. Hsp70 High Sensitivity ELISA Antibody

   10 mL, Catalog No. 80-1777

   A yellow solution of rabbit polyclonal antibody specific for Hsp70

6. Hsp70 High Sensitivity ELISA Conjugate

   10 mL, Catalog No. 80-1778

   A blue solution of goat anti-rabbit IgG conjugated to horseradish peroxidase

Protect substrate from prolonged exposure to light

7. TMB Substrate

   10 mL, Catalog No. 80-0350

   A solution of 3,3’,5,5’ tetramethylbenzidine (TMB) and hydrogen peroxide

Stop solution is caustic.

8. Stop Solution 2

   10 mL, Catalog No. 80-0377

   A 1N solution of hydrochloric acid in water

9. Hsp70 High Sensitivity Assay Layout Sheet

   1 each, Catalog No. 30-0249

10. Plate Sealer

    3 each, Catalog No. 30-0012

Reagents require separate storage conditions.

STORAGE

All components of this kit except the standard are stable at 4˚C. The standard must be stored at or below -20˚C

MATERIALS NEEDED BUT NOT SUPPLIED

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

REAGENT PREPARATION

Bring all reagents except the standard and assay buffer to room temperature for at least 30 minutes prior to opening.

1. **Wash Buffer**

   Prepare the wash buffer by diluting 50 mL of the supplied Wash Buffer Concentrate with 950 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

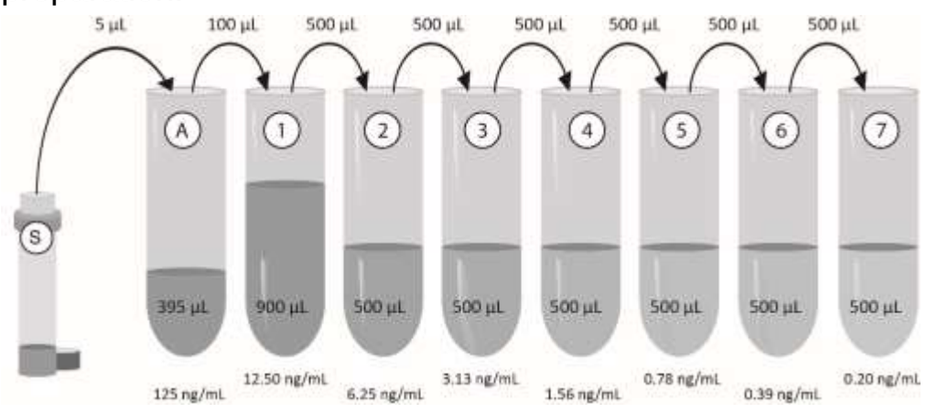
2. **125ng/ml Hsp70 Intermediate Standard**

   Label one 12x75 mm polypropylene tube as A. Pipet 400 µL of the assay buffer into tube A. Remove 5 µL of the assay buffer from the tube for a final volume of 395 µL. Add 5 µL of the Hsp70 High Sensitivity Standard stock solution. Vortex gently. Keep the intermediate standard on ice for optimal performance.

Plastic tubes must be used for standard preparation.

3. **Hsp70 Standard Curve**

   The assay buffer as well as diluted standards and samples should be kept on ice and used within 60 minutes of preparation for optimal performance. If ice is not available, room temperature assay buffer may be used and the diluted standards and samples should be used within 20 minutes of preparation.



   Label seven 12x75 mm polypropylene tubes #1 through #7. Pipet 900 µL of the assay buffer into tube#1. Pipet 500 µL of the assay buffer into tubes #2 through #7. Add 100 µL of the 125 ng/mL Hsp70 Intermediate Standard from tube A into tube #1. Vortex gently. Add 500 µL of tube #1 to tube#2 and vortex gently. Continue this for tubes #3 through #7.

SAMPLE HANDLING

If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

This assay is suitable for measuring Hsp70 (Hsp72) 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.

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. Below are examples of spike and recovery experiments with human serum and EDTA plasma samples. The samples were spiked at 3 different levels of the supplied standard (10, 5, and 0.5ng/ml) and diluted 4-fold into the assay buffer. Percent recoveries were calculated as a ratio of observed to expected levels.

Samples must be stored at or below -20°C to avoid loss of bioactive analyte. Avoid repeated freeze/ thaw cycles.

Sample	% Recovery	Recommended Dilution
Serum (human) spiked with high, medium and low levels of Hsp70	94.2	1:4
Plasma (human) spiked with high, medium and low levels of Hsp70	79.3	1:4

Diluted standards and samples should be kept on ice and used within 60 minutes of preparation for optimal performance. If ice is not available, diluted standards and samples should be used within 20 minutes of preparation.

Parallelism

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

Bring all reagents except the standard and assay buffer to room temperature for at least 30 minutes prior to opening.

All standards and samples should be run in duplicate.

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.

Pipet the reagents to the sides of the wells to avoid possible contamination.

Prior to the addition of antibody, conjugate or substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.

Serum and Plasma Preparation

1. Collect whole blood in either clotting tubes for serum or EDTA tubes for plasma.
2. Allow serum to clot for 30 minutes.
3. Centrifuge at 1000 x g for 15 minutes at 4°C.
4. Place supernatants in a clean tube.
5. The supernatant may be aliquotted and stored at or below -20°C, or used immediately in the assay.

ASSAY PROCEDURE

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with the desiccant, to the mylar bag and seal. Store unused wells at 4°C.

1. Pipet 100 µL of the assay buffer into the S0 (0 ng/mL standard) wells.
2. Pipet 100 µL of Standards #1 through #7 to the bottoms of the appropriate wells.
3. Pipet 100 µL of the samples to the bottoms of the appropriate wells.
4. Seal the plate. Incubate for 2 hours shaking* at room temperature.
5. Empty the contents of the wells and wash by adding 400 µL of wash buffer to every well. Repeat 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.
6. Pipet 100 µL of yellow antibody into each well except the blank.
7. Seal the plate. Incubate for 1 hour shaking* at room temperature.
8. Wash as above (Step 5).
9. Add 100 µL of blue conjugate to each well except the blank.
10. Seal the plate. Incubate for 1 hour shaking* at room temperature.
11. Wash as above (Step 5).
12. Pipet 100 µL of substrate solution into each well.
13. IIncubate for 30 minutes shaking* at room temperature.
14. Pipet 100 µL of stop solution into each well.

15. After zeroing the plate reader against the substrate blank, read optical density at 450nm. If plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

    *Shaking is preferably carried out on a suitable plate or orbital shaker set at a speed to ensure adequate mixing of the contents of the wells. The optimal speed for each shaker will vary and may range from 120-700rpm.

CALCULATION OF RESULTS

Multiply sample concentrations by the dilution factor used during sample preparation.

Several options are available for calculating the concentration of Hsp70 in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4-parameter logistic curve fitting program. If data reduction software is not readily available, the concentrations may be calculated as follows:

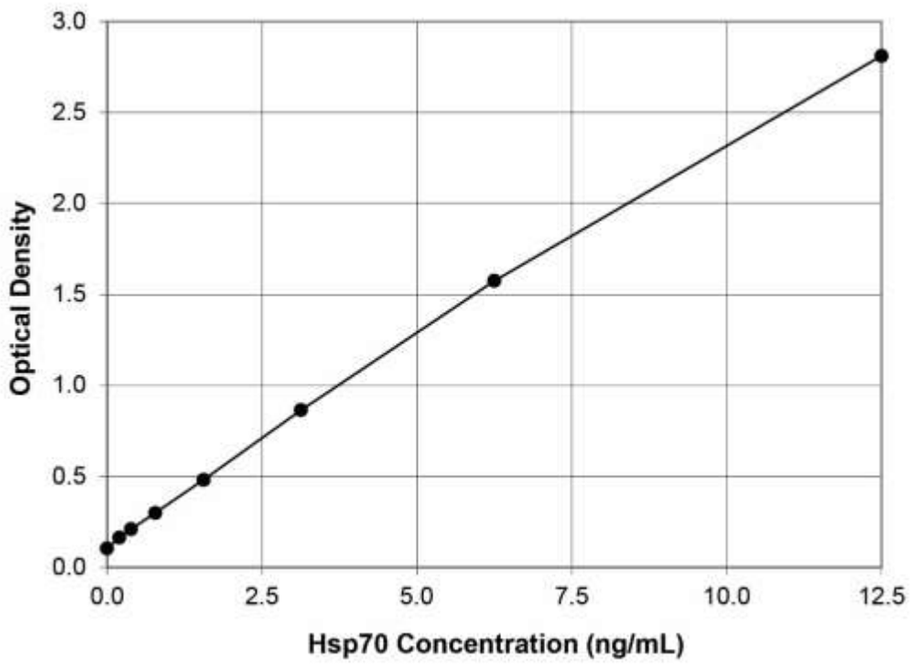
1. Calculate the average Net OD for each standard and sample by subtracting the average blank OD from the average OD for each standard and sample.
   Average Net OD = Average OD - Average Blank OD
2. Plot the average Net OD for each standard versus Hsp70 concentration in each standard. Approximate a straight line through the points. The concentration of the unknowns can be determined by interpolation.

Samples with concentrations outside of the standard curve range will need to be re-analyzed using a different dilution.

TYPICAL RESULTS

The results shown below are for illustration only and should not be used to calculate results from another assay.

Sample	Net OD	Hsp70 (ng/mL)
S0	0.104	**0**
S1	2.810	**12.50**
S2	1.574	**6.25**
S3	0.864	**3.13**
S4	0.481	**1.56**
S5	0.299	**0.78**
S6	0.211	**0.39**
S7	0.163	**0.20**
Unknown 1	1.291	**4.98**
Unknown 2	0.372	**1.08**



PERFORMANCE CHARACTERISTICS

Specificity

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
Hsp70 (human)	100%
Hsp70 (rat)	117.6%
Hsp70B' (human)	5.4%
Hsp70 (salmon)	0.8%
DnaK (*E.coli*)	0.5%
Hsc70 (bovine)	<0.016%
Grp78 (hamster)	<0.016%
Hsp71 (*M. tuberculosis*)	<0.016%

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

A buffer sample containing Hsp70 was serially diluted 1:2 in assay buffer 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

Precision

Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing Hsp70 in a single assay.

ng/mL	%CV
5.11	3.9
2.19	11.4
0.99	5.9

Inter-assay precision was determined by measuring buffer controls of varying Hsp70 concentrations in multiple assays over several days.

ng/mL	%CV
4.98	12.8
2.63	13.7
1.08	19.1

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