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# ab222879 Human Heat Shock Factor Protein (HSF 1) ELISA Kit

For the quantitative measurement of human HSF 1 in cell lysate and tissue extract samples.

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

# Table of Contents

# 1. Overview

The Human HSF 1 ELISA (Enzyme-Linked Immunosorbent Assay) kit (ab222879) is designed for detection of human HSF 1 in cell lysate and tissue extract samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human HSF 1 in approximately 4 hours. A polyclonal antibody specific for human HSF 1 has been pre-coated onto a 96-well microplate with removable strips. HSF 1 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human HSF 1, which is recognized by a streptavidin-peroxidase (SP) conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Heat shock factor protein 1 (HSF 1), also known as heat shock transcription factor 1 or HSTF 1, is a 529-amino acid transcription factor of 57 kDa. HSF 1 is present in unstressed cells as an inactive monomeric form and becomes activated by heat and other stress stimuli. HSF 1 is rapidly induced after temperature stress and binds heat shock promoter elements (HSE) that are present upstream of all the heat shock genes. Heat shock protein 90 (HSP90) inhibits HSF 1 activation. HSP90-containing HSF 1 complex is present in the unstressed cell and dissociates during stress. HSF 1 plays a crucial role in inducing heat shock proteins, which is required for thermotolerance. It is involved in oogenesis, spermatogenesis, placental development, and regulation of lifespan.

## 2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells and incubate for 2 hours

Wash wells, add 50 µL Biotinylated Antibody to each well and incubate for 1 hour

Wash wells, add 50 µL Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes

Wash wells, add 50 µL Chromogen Substrate to each well and incubate for 30 minutes

Add 50 µL Stop Solution to each well and read OD at 450 nm

## 3. Precautions

**Please read these instructions carefully prior to beginning the assay.**

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

## 4. Storage and Stability

**Store kit at 4°C immediately upon receipt, apart from Biotinylated Human HSF 1, Human HSF 1 Standard and 100X Streptavidin-Peroxidase Conjugate which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

## 5. 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.

## 6. Materials Supplied

| Item | Quantity | Storage Condition |
|---|---|---|
| Anti-Human HSF 1 coated Microplate (12 x 8 wells) | 96 wells | +4°C |
| Human HSF 1 Standard | 1 Vials | -20°C |
| Biotinylated Human HSF 1 | 150 µl | -20°C |
| 10X Diluent N Concentrate | 30 mL | +4°C |
| 1X Standard Diluent | 2 mL | +4°C |
| 20X Wash Buffer Concentrate | 2 x 30 mL | +4°C |
| 100X Streptavidin-Peroxidase Conjugate | 80 µl | -20°C |
| Chromogen Substrate | 7 mL | +4°C |
| Stop Solution | 11 mL | +4°C |
| Sealing Tapes | 3 units | +4°C |

## 7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µL, 20-200 µL, 200-1000 µL, and multiple channel).
- Deionized or distilled reagent grade water.

## 8. Technical Hints

- 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.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

# 9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare only as much reagent as is needed on the day of the experiment.

### 9.1 10X Diluent N Concentrate:

If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Prepare 1X Diluent N by diluting Diluent N Concentrate 1 in 10 with reagent grade water to produce a 1X solution. Store for up to 30 days at 2-8°C.

### 9.2 Biotinylated Human HSF 1:

Spin down the antibody briefly and dilute the desired amount of the antibody 1 in 40 with 1X Diluent N to produce a 1X solution. The undiluted antibody should be stored at -20°C.

### 9.3 20X Wash Buffer Concentrate:

If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Prepare 1X Wash Buffer by diluting Wash Buffer Concentrate 1 in 20 with reagent grade water.

### 9.4 100X Streptavidin-Peroxidase Conjugate:

Spin down the Streptavidin-Peroxidase Conjugate briefly and dilute the desired amount of the conjugate 1 in 100 with 1X Diluent N. The undiluted conjugate should be stored at -20°C.

### 9.5 Standard Diluent (2 mL):

Ready to use. Store at +4°C.

### 9.6 Chromogen Substrate (8 mL):

Ready to use. Store at +4°C.

### 9.7 Sealing Tapes (3 units):

Ready to use. Store at +4°C.

### 9.8 Stop Solution (12 mL):

Ready to use. Store at +4°C.

## 10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

**10.1** Reconstitute the HSF 1 to generate a 40 ng/mL **Stock**.

10.1.1 First consult the HSF 1 Standard vial to determine the mass of protein in the vial.

10.1.2 Calculate the appropriate volume of 1X Diluent N to add when resuspending the HSF 1 Standard vial to produce a 40 ng/mL HSF 1 **Stock** by using the following equation:

CS = Starting mass of HSF 1 Standard stock (see vial label) (ng)

CF = 40 ng/mL HSF 1 Standard #1 final required concentration

VD = Required volume of 1X Diluent N for reconstitution (µL)

Calculate total required volume 1X Diluent N for resuspension:

$$(C_S / C_S) * 1{,}000 = V_D$$

Example:

**NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.**

$C_S$ = 12 ng of HSF 1 Standard in vial

$C_F$ = 40 ng/mL HSF 1 stock final concentration

$V_D$ = Required volume of 1X Diluent N for reconstitution

(12 ng / 40 ng/mL) * 1,000 = 300 µL

10.1.3 First briefly centrifuge the HSF 1 Standard Vial to collect the contents on the bottom of the tube.

10.1.4 Reconstitute the HSF 1 Standard vial by adding the appropriate calculated amount VD of 1X Diluent N to the vial to generate the 40 ng/mL HSF 1 **Stock.** Mix gently and thoroughly.

**10.2** Allow the reconstituted 40 ng/mL HSF 1 **Stock** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions

**10.3** Label seven tubes #1 – 7.

**10.4** Prepare duplicate or triplicate standard points by serially diluting the **Stock** solution (40 ng/mL) 1 in 2 with 1X Diluent N to produce 20, 10, 5, 2.5, 1.25, and 0.625 ng/mL solutions. 1X Diluent N serves as the zero standard (0 ng/ml).

**10.5** Add 120 µL of 1X Diluent N to tube #1 – 7.

**10.6** To prepare **Standard #1**, add 120 µL of the **Stock** into tube #1 and mix gently.

**10.7** To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.

**10.8** Using the table below as a guide, prepare subsequent serial dilutions.

| Standard # | Volume to dilute (µL) | Volume Diluent N (µL) | HSF 1 (ng/mL) |
|---|---|---|---|
| 1 | 120 µL Stock from Step 10.1 | 120 | 20 |
| 2 | 120 µL Standard #1 | 120 | 10 |
| 3 | 120 µL Standard #2 | 120 | 5 |
| 4 | 120 µL Standard #3 | 120 | 2.5 |
| 5 | 120 µL Standard #4 | 120 | 1.25 |
| 6 | 120 µL Standard #5 | 120 | 0.625 |
| 7 (Blank) | N/A | 120 | 0 |

# 11. Sample Preparation

## 11.1 Cell Lysate:

Rinse cell with cold PBS and then scrape the cell into a tube with 5 ml of cold PBS and 0.5 M EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4°C and aspirate supernatant. Re-suspend pellet in ice-cold Lysis Buffer (10 mM Tris, pH 8.0, 130 mM NaCl, 1% Triton X-100, protease inhibitor cocktail). For every 1 x $10^6$ cells, add approximately 100 µL of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 rpm for 30 minutes at 4°C and collect supernatant.

## 11.2 Tissue Extract:

Extract tissue samples with 0.1 M phosphate-buffered saline (pH 7.4) containing 1% Triton X-100 and centrifuge at 14000 x g for 20 minutes. Collect the supernatant and measure the protein concentration. Freeze remaining extract at -20°C or below.

## 12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

12.1 Prepare all reagents, working standards, and samples as directed in the previous sections

12.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and store in a vacuum desiccator.

12.3 Add 50 µL of all sample or standard to appropriate wells. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.

12.4 Wash five times with 200 µL of Wash Buffer manually. Wash by aspirating or decanting from wells then dispensing 200 µL Wash Buffer into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid. If using a machine, wash six times with 300 µl of Wash Buffer.

12.5 Add 50 µL of 1X Biotinylated Human HSF 1 to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed and incubate for 1 hour.

12.6 Wash the microplate as described above (12.4).

12.7 Add 50 µL of 1X Streptavidin-Peroxidase Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes.

12.8 Turn on the microplate reader and set up the program in advance.

12.9 Wash the microplate as described above (12.4).

12.10 Add 50 µL of Chromogen Substrate per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed and incubate for 30 minutes or till the optimal blue color density develops.

12.11 Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.

12.12 Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only.

**Δ Note:** that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

## 13. Calculations

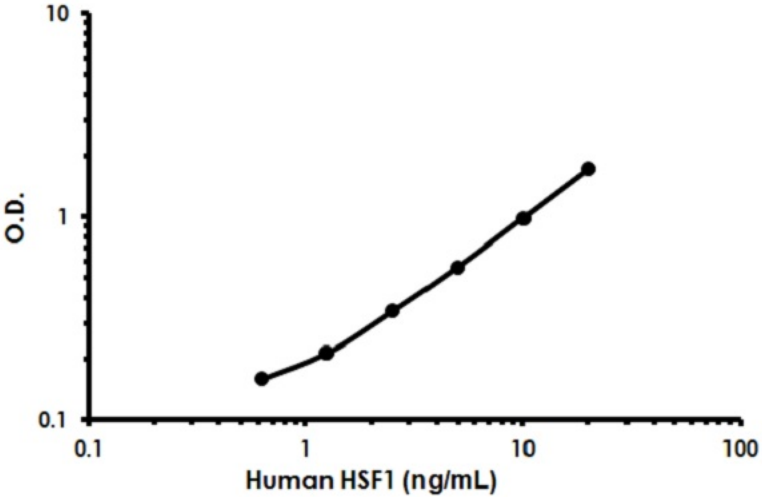
13.1 Calculate the mean value of the duplicate or triplicate readings for each standard and sample.

13.2 To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using four-parameter or log-log logistic curve-fit.

13.3 Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

## 14. Typical Data

**Typical standard curve** – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.




| Standard Curve Measurements | | | |
|---|---|---|---|
| Concentration (ng/mL) | O.D 450 nm | | Mean O.D |
| | 1 | 2 | |
| 0 | 0.088 | 0.096 | 0.092 |
| 0.625 | 0.155 | 0.163 | 0.159 |
| 1.25 | 0.212 | 0.216 | 0.214 |
| 2.5 | 0.337 | 0.349 | 0.343 |
| 5 | 0.540 | 0.594 | 0.567 |
| 10 | 0.981 | 0.993 | 0.987 |
| 20 | 1.670 | 1.764 | 1.717 |

**Figure 1**. Example of human HSF 1 standard curve in 1X Diluent N. The HSF 1 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

## 15. Typical Sample Values

### SENSITIVITY –

The calculated minimal detectable dose (MDD) is 0.5 ng/mL. The MDD was determined by calculating the mean of zero standard and adding 2 standard deviations then extrapolating the corresponding concentration.

### PRECISION –

Intra-assay precision was determined by testing three internal plasma controls twenty times in one assay.

Inter-assay precision was determined by testing three internal plasma controls in twenty assays.

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| Sample | 1 | 2 | 3 | 1 | 2 | 3 |
| n | 20 | 20 | 20 | 20 | 20 | 20 |
| CV (%) | 4.9% | 5.4% | 4.5% | 10.1% | 9.4% | 9.8% |
| Average CV (%) | 4.9% | | | 9.8% | | |

## 16. Assay Specificity

This kit recognizes human HSF 1 protein in cell lysate and tissue extract.

### CROSS REACTIVITY

| Species | Cross Reactivity (%) |
|---|---|
| Dog | 70% |
| Cow | 20% |
| Monkey | 100% |
| Mouse | None |
| Rat | 50% |
| Pig | 100% |
| Rabbit | None |

## 17. Species Reactivity

This kit recognizes human, dog, cow, monkey, rat and pig HSF 1 protein.

Please contact our Technical Support team for more information.

# 18. Troubleshooting

| Problem | Reason | Solution |
|---|---|---|
| Low Precision | Use of expired components | Check the expiration date listed before use. Do not interchange components from different lots. |
| | Improper wash step | Check that the correct wash buffer is being used. Check that all wells are dry after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique. |
| | Splashing of reagents while loading wells | Pipette properly in a controlled and careful manner. |
| | Inconsistent volumes loaded into wells | Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance. |
| | Insufficient mixing of reagent dilutions | Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions. |
| | Improperly sealed microplate | Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing. |
| **Unexpectedly Low or High Signal Intensity** | Microplate was left unattended between steps | Each step of the procedure should be performed uninterrupted. |
| | Omission of step | Consult the provided procedure for complete list of steps. |
| | Steps performed in incorrect order | Consult the provided procedure for the correct order. |
| | Insufficient amount of reagents added to wells | Check pipette calibration. Check pipette for proper performance. |
| | Wash step was skipped | Consult the provided procedure for all wash steps. |
| | Improper wash | Check that the correct wash buffer |

| | | |
|---|---|---|
| | buffer | is being used. |
| | Improper reagent preparation | Consult reagent preparation section for the correct dilutions of all reagents. |
| | Insufficient or prolonged incubation periods | Consult the provided procedure for correct incubation time. |
| **Deficient Standard Curve Fit** | Non-optimal sample dilution | Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. User should determine the optimal dilution factor for samples. |
| | Contamination of reagents | A new tip must be used for each addition of different samples or reagents during the assay procedure. |
| | Contents of wells evaporate | Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature. |
| | Improper pipetting | Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance. |
| | Insufficient mixing of reagent dilutions | Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions. |

# 19. Notes

## Technical Support



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