ELISA PRODUCT INFORMATION & MANUAL

HSF1

NBP2-62149

Enzyme-linked Immunosorbent Assay for quantitative detection of Human HSF1.
For research use only.
Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

HSF1 ELISA kit

Catalog # **NBP2-62149**

96 Well Enzyme Immunoassay Kit
For use with cell lysates and tissue extracts

Reagents require separate storage conditions.

Check our website for additional protocols, technical notes and FAQs.

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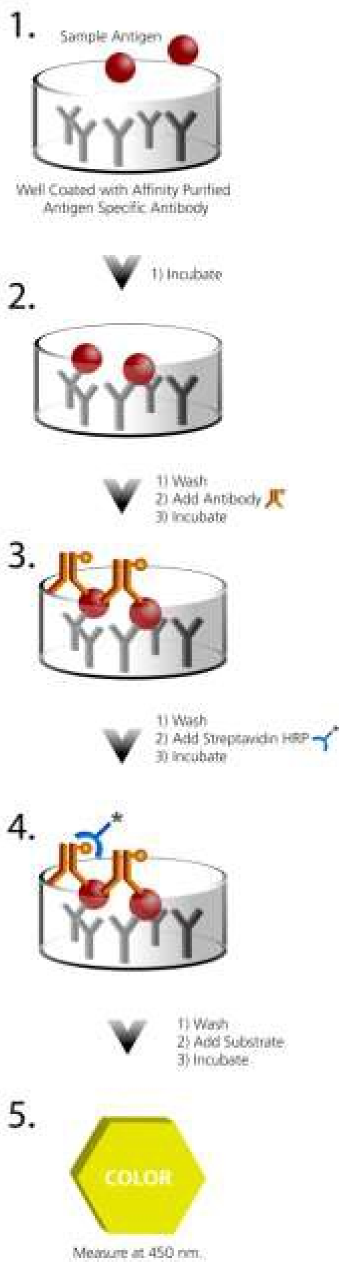


Introduction

The HSF1 ELISA kit is a complete kit for the quantitative determination of HSF1 in cell lysates and tissue extracts from human and mouse origins. Please read the complete kit insert before performing this assay.

HSF1 belongs to a family of Heat Shock transcription Factors (HSFs) that bind to highly conserved heat shock elements (HSEs) in the promoter regions of heat shock genes and regulate the expression of heat shock proteins (HSPs)[1]. Most HSFs have several common functional motifs including an N-terminal DNA binding domain essential for binding to the HSE and adjacent hydrophobic repeats essential for the formation of active trimers.

Another short hydrophobic repeat located towards the C-terminal region of most HSFs is thought to be necessary for suppression of trimerization except in the case of yeast HSF and human HSF4. In higher eukaryotes, HSF1 is predominantly found in a diffuse cytoplasmic and nuclear distribution in unstressed cells. On exposure to heat shock and other stresses, HSF1 localizes within seconds to discrete nuclear granules and on recovery from stress, HSF1 rapidly dissipates from the stress granules to a diffuse nucleoplasmic distribution[2]. HSF1 is post-translationally modified by both phosphorylation and SUMOylation. Inducible phosphorylation at Ser^{230}, Ser^{326}, and Ser^{419} promote HSF1 activity, while constitutive phosphorylation sites at Ser^{303}, Ser^{307}, and Ser^{308} are inhibitory[3-5].

Principle

1. Samples and standards are added to wells coated with a rat monoclonal antibody to HSF1. The plate is then incubated.
2. The plate is washed, leaving only bound HSF1 on the plate. A yellow solution of biotinylated polyclonal antibody to HSF1 is then added. This binds to the HSF1 captured on the plate. The plate is then incubated.
3. The plate is washed to remove excess biotinylated antibody. A blue solution of streptavidin conjugated to horseradish peroxidase is added to each well, binding to the biotinylated polyclonal HSF1 antibody.
4. The plate is washed to remove excess HRP conjugate. TMB Substrate solution is added. The HRP-catalyzed reaction generates a blue color in the solution.
5. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450 nm. The amount of signal is directly proportional to the level of HSF1 in the sample.

Materials Supplied

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

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

1. **Assay Buffer 29**
   100 mL
   Tris buffered saline containing BSA and detergents

2. **HSF1 Standard**
   0.5 mL
   2 vials, each vial containing a solution of 25ng/mL recombinant human HSF1.

3. **HSF1 Clear Microtiter Plate**
   One plate of 96 wells
   A clear plate of break-apart strips coated with a monoclonal antibody specific for HSF1

4. **Biotinylated HSF1 Antibody**
   10 mL
   A yellow solution of biotinylated polyclonal antibody to HSF1

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

5. **HSF1 Conjugate**
   10 mL
   A blue solution of streptavidin conjugated to horseradish peroxidase

6. **Wash Buffer Concentrate**
   100 mL
   Tris buffered saline containing detergents

Protect substrate from prolonged exposure to light.

7. **TMB Substrate**
   10 mL
   A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide

Stop solution is caustic. Keep tightly capped.

8. **Stop Solution 2**
   10 mL
   A 1N solution of hydrochloric acid in water

9. **HSF1 Assay Layout Sheet**
   1 each

10. **Extraction Reagent #1**
    10 mL
    A concentrated cell/tissue lysis buffer.

11. **Plate Sealer**
    3 each

Storage

Reagents require separate storage conditions.

All components of this kit, **except the standard and microtiter plate**, are stable at 4°C until the kit's expiration date. The standard and microtiter plate **must** be stored at -20°C upon receipt. All components may be stored at -20°C with up to 3 freeze/thaw cycles.

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
5. Graduated cylinders
6. A microplate shaker
7. Lint-free paper for blotting
8. Microplate reader capable of reading 450 nm
9. Software for extrapolating sample values from optical density readings utilizing a four parameter logistic curve fit.
10. Protease Inhibitor Cocktail (PIC)
11. Phosphatase Inhibitor Cocktail (PhIC)
12. Hank's Balanced Salt Solution without phenol red, or PBS
13. 12x75 mm test tubes
14. Microcentrifuge tubes

Reagent Preparation

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

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

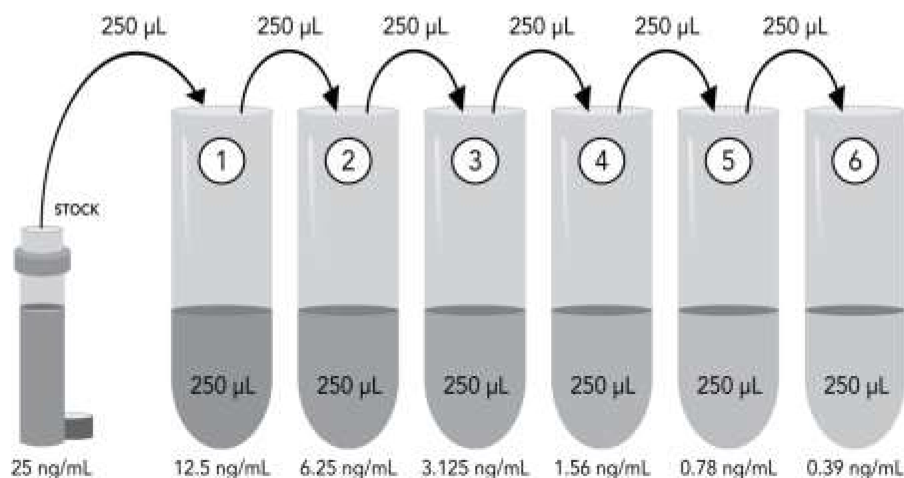
The standard should be handled with care due to the known and unknown effects of the antigen.

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's expiration date, or for 3 months, whichever comes first.

2. **Extraction Reagent**
   Prepare the extraction reagent by dilution 1 mL of the supplied concentrate with 4 mL of deionized water.

3. **Addition of Inhibitors to Extraction Reagent** Immediately prior to use, PIC and PhIC must be added to the 1x extraction reagent. Add 0.5 µl/mL of Protease Inhibitor Cocktail or equivalent concentration according to vendor's specification sheet.
   Note: Inhibitors must be freshly added to Extraction Reagent to ensure optimal integrity of the samples. Buffers containing inhibitors should be used within 1 hour of preparation. **Do not use PMSF. PMSF interferes with this assay.**

4. **Preparation of HSF1 Standard Curve**
   Thaw the standard on ice. Vortex, then briefly centrifuge contents to the bottom of vial.
   Label six disposable 12 x 75 mm tubes #1 through #6. Pipet 250 µL standard diluent into each tube. Add 250 µL of the standard stock to tube #1 and vortex thoroughly. Remove 250 µL from tube #1 and add to tube #2. Vortex thoroughly. Continue this for tubes #3 through #6.



Diluted standards should be used within 1 hour of preparation. The concentrations of the standards are labeled above.

Sample Handling

Samples must be stored frozen at or below -20° to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.

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 HSF1 in cell and tissue lysates of human and mouse origin. Prior to assay, frozen samples should be slowly brought to 4°C and centrifuged, if necessary, to isolate residual cell debris.

A minimum 1:4 dilution of the samples is required to remove matrix interference of Extraction Reagent #1. Due to differences in cell types, number of cells, or total cellular protein concentration, lysates may require greater dilution with the assay buffer to remove interference or to be read within the standard range. Below are examples of lysates that have been run in this assay.

Cell Lysate Sample	Total cellular protein (mg/ml)	% Recovery*	Recommended Dilution
3T3	6	100	1:4
3T3 heat-shocked	4.4	107	1:4
HeLa	2.5	100	1:4
HeLa heat-shocked	2.15	105	1:4

*%Recovery based on dilutional linearity.

Sample Preparation

Protocol for Cell Lysis

1. Harvest cells and centrifuge at 250 x g for 7 minutes at 4°C. Discard supernatant.
2. Resuspend pellet and wash with Hank's Balanced Salt Solution (without phenol red) or PBS.
3. Centrifuge at 250 x g for 7 minutes at 4°C. Discard supernatant.
4. Resuspend pellet with Extraction Reagent #1 plus protease and phosphatase inhibitors (see Reagent Preparation section).
5. Vortex and incubate on ice for 30 minutes.
6. Centrifuge at 16,000 x g for 20 minutes at 4°C.
7. Place the supernatants into a clean tube.
8. The supernatants may be aliquoted and stored at or below -20°C or used immediately in the assay.

Protocol for Tissue Extracts

1. Harvest tissue to be analyzed.
2. If necessary, tissues can be flash frozen, stored at -70°C and the extract prepared at a later time.
3. Calculate the amount of cold 1X Extraction Reagent that will be required. For each 0.5 cm^3 piece of tissue, use 1ml of 1X Extraction Reagent.
4. Add protease and phosphatase inhibitors to the 1X Extraction Reagent (see Reagent Preparation section).
5. Place the tissue in a mortar and add a sufficient volume of liquid nitrogen to the cover the tissue sample.
6. Allow the liquid nitrogen to evaporate. The tissue should be thoroughly frozen.
7. Grind the frozen tissue to a powder with a pestle.
8. Add an appropriate amount of cold 1X Extraction Reagent supplemented with inhibitors to the processed tissue.
9. Continue to homogenize the tissue with the pestle until the tissue suspension is homogeneous.
10. Transfer the extract to a polypropylene tube and centrifuge at 21,000 X g for 10 minutes in a 4°C refrigerated microfuge.
11. Transfer the supernatant to a labeled polypropylene tube. The supernatant collected is the tissue extract, which is now ready for analysis using the HSF1 ELISA kit. The resulting pellet may be discarded.
12. Alternatively, the tissue extracts can be frozen at -70°C and assayed at a later date. It is recommended that a protein determination assay be performed and the extracts aliquotted to convenient amounts prior to storing at -70 °C to avoid multiple freeze thaw cycles.

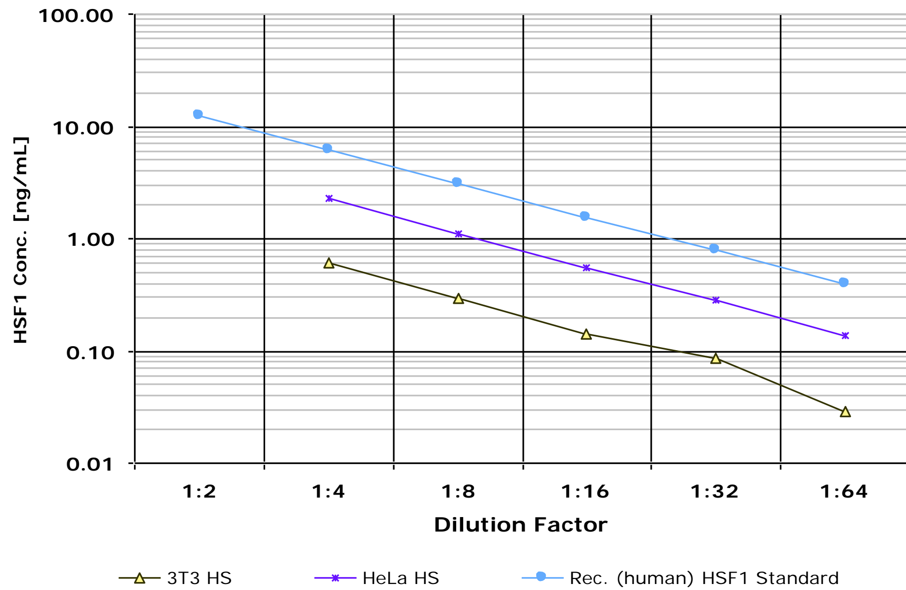
Dilutional Linearity

The minimum required dilution for several common samples was determined by serially diluting samples into the assay buffer and identifying the dilution at which linearity was observed.

Cell Lysate:	HeLa heat shocked	Cell Lysate:	3T3 heat shocked
Dilution	% Linearity	Dilution	% Linearity
1:4	105	1:4	107
1:8	99	1:8	103
1:16	100	1:16	100
1:32	103	1:32	119

Parallelism

Parallelism experiments were carried out to determine if the recombinant human HSF1 standard accurately determines human HSF1 concentrations in biological matrices. Values were obtained using the cell supernatants from treated cultures serially diluted in assay buffer and assessed from a standard curve using four parameter logistic curve fitting. The observed values were plotted against the dilution factors. Parallelism of the curves demonstrates that the antigen binding characteristics are similar enough to allow the accurate determination of native analyte levels in diluted samples.



Assay Procedure

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

Pipet standards and samples to the bottom of the wells.

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, and substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.

Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the foil pouch and seal the ziploc. Store unused wells at -20°C.

1. Pipet 100 µL of Assay Buffer 29 into the S0 (0 pg/mL standard) wells.
2. Pipet 100 µL of Standards #1 through #6 into the appropriate wells.
3. Pipet 100 µL of the Samples into the appropriate wells.
4. Seal the plate. Incubate at room temperature on a plate shaker for 1 hour at ~500 rpm*.
5. Empty the contents of the wells and wash by adding 400 µL of wash solution 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.
6. Pipet 100 µL of yellow biotinylated Antibody into each well, except the Blank.
7. Seal the plate. Incubate at room temperature on a plate shaker for 1 hour at ~500 rpm*.
8. Wash as above (Step 5).
9. Add 100 µL of blue Conjugate to each well, except the Blank.
10. Seal the plate. Incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm*.
11. Wash as above (Step 5). Pipet 100 µL of Substrate Solution into each well.
12. Seal the plate. Incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm*.
13. Pipet 100 µL Stop Solution to each well.
14. Zero the plate reader against the Blank wells, read the optical density at 450 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all the readings.

* The actual speed of the plate shaker should be such that the liquid in the plate wells mixes thoroughly, but does not splash out of the well.

Calculation of Results

Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

Several options are available for the calculation of the concentration of HSF1 in the samples. We recommend that the data be handled by an immunoassay software package utilizing a four parameter logistic curve fitting program. Please note that such software is often supplied by plate reader manufacturers. If data reduction software is not readily available, the concentration of HSF1 can be calculated by hand 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 Blanks OD

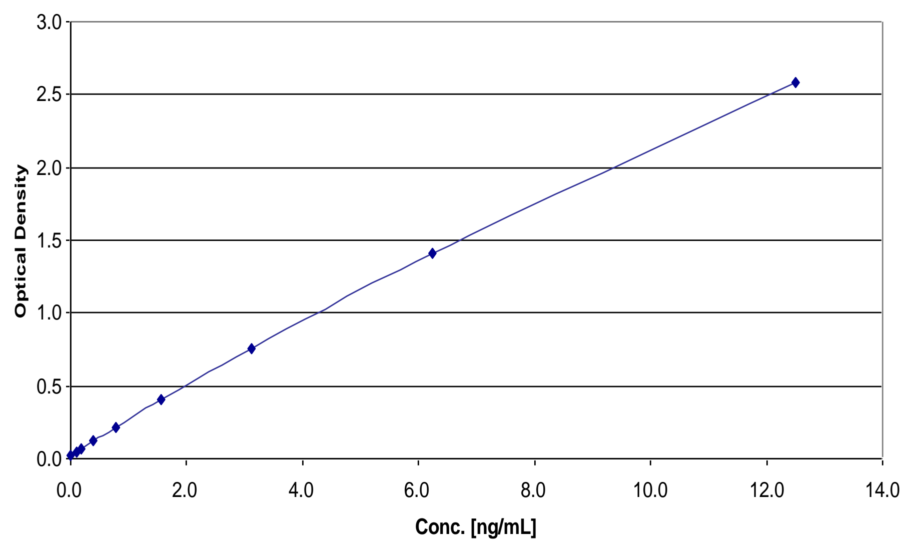
2. Using linear graph paper, plot the average Net OD for each standard versus HSF1 concentration in each standard. Approximate a straight line through the points. The concentration of the unknowns can be determined by interpolation.

Samples with concentrations outside of the standard curve range will need to be reanalyzed 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	HSF1 (ng/mL)
S0	0.00	0.023
S1	12.5	2.588
S2	6.25	1.408
S3	3.125	0.757
S4	1.56	0.409
S5	0.78	0.120
S6	0.39	0.071



Performance Characteristics

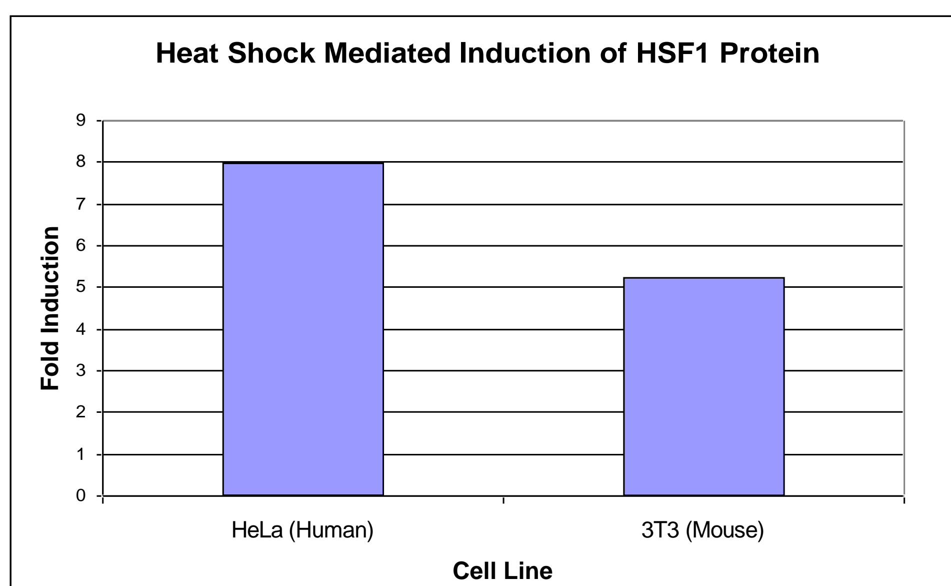
Specificity

The cross reactivity of HSF1 with HSF2 and HSF4 was determined by diluting it in the assay buffer at several concentrations. Cross reactivity was then measured in the assay.

Compound	Cross Reactivity
HSF2	<0.023 %
HSF4	< 0.012 %

Stimulation Experiments

HeLa and 3T3 cells were grown to approximately 80% confluency and subjected to heat shock at 42°C for 2 hours. Extracts were prepared as described in “Sample Preparation” and the levels of HSF1 were determined in the assay. Induction is expressed as the fold change relative to non heat-shocked control samples.



Sensitivity

The sensitivity or limit of detection of the assay is 35 pg/mL. The sensitivity was determined by interpolation at 2 standard deviations above the mean signal at background (0 ng/mL) using data from 6 standard curves.

Precision

Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing HSF1 in a single assay.

ng/ml	%CV
8.38	1.161
4.8	1.464
2.68	2.898

Inter-assay precision was determined by measuring buffer controls of varying HSF1 concentrations in multiple assays over several days.

ng/ml	%CV
9.12	7.19
4.9	4.96
2.53	4.9

References

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3. Holmberg, C.I., *et al*. (2002) Trends Biochem Sci. **27**, 619-627.
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Notes

Notes

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