# abcam

# Product datasheet

# Anti-HSF1 antibody ab2923

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#### Overview

Product name Anti-HSF1 antibody

**Description** Rabbit polyclonal to HSF1

Host species Rabbit

Tested applications Suitable for: IP, WB, ICC/IF

Species reactivity Reacts with: Mouse, Human, Caenorhabditis elegans

Immunogen Recombinant full length protein corresponding to Human HSF1. Recombinant human HSF1

expressed in E. coli.

Positive control WB: HEK-293T, HeLa, K562, A431, and HepG2 whole cell lysate. C. elegans whole worm tissue.

ICC/IF: HeLa and NIH3T3 cells. IP: HeLa whole cell lysate.

**General notes**The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

#### **Properties**

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.05% Sodium azide

Constituent: 99% PBS

**Purity** Whole antiserum

Primary antibody notes

All organisms respond to elevated temperatures and a variety of environmental stresses by rapid

synthesis of heat shock RNAs and proteins. The regulation of heat shock gene transcription is mediated by the transcriptional activator, heat shock factor (HSF), which binds to heat shock response elements (HSEs). These HSEs are found as three repeats of a 5-nucleotide {nGAAn} module, arranged in alternating orientation and present upstream of all heat shock genes. The HSEs are highly conserved among species yet HSF purified from yeast, Drosophila and human

have different molecular weights and the proteins do not show significant immunological cross reaction. Two HSFs have been identified in human cells, HSF 1 and HSF 2, which bind to the same HSEs and have 38% sequence identity. These factors are activated by distinct stimuli, HSF 1 is responsive to classical stress signals such as heat, heavy metals and oxidative reagents, whereas HSF 2 is activated during hemin-mediated differentiation of human erythroleukemia cells. HSF 1 exists constitutively in the cytoplasm and the nucleus of unstressed cells as a monomer which lacks DNA binding activity. Through an unknown signal generated during stress, HSF 1 becomes activated to a nuclear localized, trimeric state which binds to DNA. The phosphorylation of HSF 1 is necessary for maximal transcription of heat shock genes.

**Clonality** Polyclonal

**Isotype** IgG

#### **Applications**

### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab2923 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IP		Use at an assay dependent concentration. 2 µL
WB	****(1)	1/1000 - 1/10000. Detects a band of approximately 83 kDa (predicted molecular weight: 57 kDa).
ICC/IF		1/50.

## **Target**

**Function** 

DNA-binding protein that specifically binds heat shock promoter elements (HSE) and activates transcription. In higher eukaryotes, HSF is unable to bind to the HSE unless the cells are heat shocked.

Sequence similarities

Belongs to the HSF family.

Domain

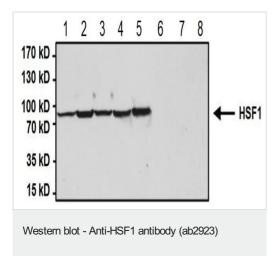
the 9aaTAD motif is a transactivation domain present in a large number of yeast and animal transcription factors.

Post-translational modifications

Phosphorylated on multiple serine residues, a subset of which are involved in stress-related regulation of transcription activation. Constitutive phosphorylation represses transcriptional activity at normal temperatures. Levels increase on specific residues heat-shock and enhance HSF1 transactivation activity. Phosphorylation on Ser-307 derepresses activation on heat-stress and in combination with Ser-303 phosphorylation appears to be involved in recovery after heat-stress. Phosphorylated on Ser-230 by CAMK2, in vitro. Cadmium also enhances phosphorylation at this site. Phosphorylation on Ser-303 is a prerequisite for HSF1 sumoylation. Phosphorylation on Ser-121 inhibits transactivation and promotes HSP90 binding. Phosphorylation on Thr-142 also mediates transcriptional activity induced by heat. Phosphorylation on Ser-326 plays an important role in heat activation of HSF1 transcriptional activity.

Sumoylated with SUMO1 and SUMO2 on heat-shock. Heat-inducible sumoylation occurs after 15 min of heat-shock, after which levels decrease and at 4 hours, levels return to control levels. Sumoylation has no effect on HSE binding nor on transcriptional activity. Phosphorylation on Ser-303 is a prerequisite for sumoylation.

#### **Images**



All lanes: Anti-HSF1 antibody (ab2923) at 1/1000 dilution

**Lane 1 :** HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

**Lane 3**: K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate

Lane 4: A431 (Human epidermoid carcinoma cell line) whole cell lysate

**Lane 5 :** HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

Lane 6 : COS-7 (African green monkey kidney fibroblast-like cell line) whole cell lysate

Lane 7: NIH/3T3 (Mouse embryo fibroblast cell line) whole cell lysate

Lane 8: NRK (Rat kidney normal tissue) whole cell lysate

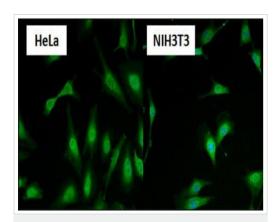
Lysates/proteins at 50 µg per lane.

#### Secondary

**All lanes :** HRP-conjugated goat anti-rabbit lgG secondary antibody at 1/20000 dilution

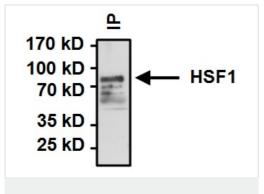
Predicted band size: 57 kDa

Western blot analysis of Heat Shock Factor 1 (HSF1) was performed by loading samples onto a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to a PVDF membrane and blocked with 5% BSA/TBST for at least 1 hour. The membrane was incubated with ab2923 overnight at 4°C on a rocking platform, washed in TBS-0.1% Tween 20, and probed with a secondary antibody for at least one hour. Chemiluminescent detection was performed.



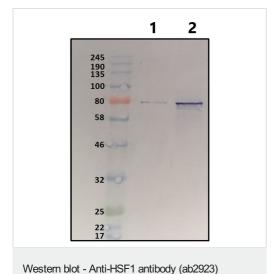
Immunocytochemistry/ Immunofluorescence - Anti-HSF1 antibody (ab2923)

Immunocytochemistry/Immunofluorescence analysis of HSF1 (green) in HeLa (Human epithelial cell line from cervix adenocarcinoma) and NIH/3T3 (Mouse embryo fibroblast cell line) cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% BSA for 15 minutes at room temperature. Cells were probed with ab2923 (1:50) for at least 1 hour at room temperature, washed with PBS, and incubated with a DyLight 488-conjugated goat-anti rabbit IgG secondary antibody (1:400) for 30 minutes at room temperature. Nuclei (blue) were stained with Hoechst 33342 dye. Images were taken at 20X magnification.



Immunoprecipitation - Anti-HSF1 antibody (ab2923)

Immunoprecipitation of HSF1 was performed on HeLa (Human epithelial cell line from cervix adenocarcinoma) cells. Antigen:antibody complexes were formed by incubating 500µg whole cell lysate with 2µg of ab2923 overnight on a rocking platform at 4°C. Immune complexes were captured on 50µl Protein A/G Plus Agarose, washed extensively, and eluted with buffer. Samples were resolved on a 4-20% Tris-HCl polyacrylamide gel, transferred to a PVDF membrane, and blocked with 5% BSA/TBST for at least 1 hour. The membrane was incubated with ab2923 (1:1000) overnight rotating at 4°C, washed in TBST, and probed with detection reagent (1:1000) for at least one hour. Chemiluminescent detection was performed.



All lanes: Anti-HSF1 antibody (ab2923) at 1/5000 dilution

Lane 1 : HSF1 RNAi treated C. elegans whole worm tissue

Lane 2: Untreated C. elegans whole worm tissue

Lysates/proteins at 10 µg per lane.

#### Secondary

**All lanes:** anti-rabbit lgG HRP-linked at 1/5000 dilution

Predicted band size: 57 kDa

Western blot analysis of HSF1 was performed by loading each sample onto an SDS-PAGE gel. Proteins were transferred to PVDF membrane and followed by blocking in TBST+5% non-fat

milk. HSF1 was detected using the primary anitbody (ab2923) in TBST+2% non-fat milk overnight at 4°C on a rocking platform, followed by secondary antibody for 30 min. Detection was performed using and 1-Step™ TMB-Blotting Substrate Solution.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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