abcam

Product datasheet

Anti-HSF1 antibody [10H8] ab61382

KO VALIDATED

13 References 3 Images

Overview

Product name	Anti-HSF1 antibody [10H8]
Description	Rat monoclonal [10H8] to HSF1
Host species	Rat
Specificity	Detects ~85kDa (unstressed cell lysates), and~95kDa (heat shocked cell lysates).
Tested applications	**Suitable for:** IHC-P, Flow Cyt, WB
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat, Rabbit, Guinea pig, Hamster, Cow, Monkey
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1, HeLa and K562 cell lysates. Flow Cyt: HeLa cells. IHC-P: Human testis tissue.
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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.09% Sodium azide Constituents: PBS, 50% Glycerol
Purity	Protein G purified
Clonality	Monoclonal
Clone number	10H8
Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab61382 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
IHC-P		Use a concentration of 5 µg/ml.
Flow Cyt		Use 1µg for 10^6 cells. **ab18407** - Rat monoclonal IgG1, is suitable for use as an isotype control with this antibody.
WB		1/1000. Predicted molecular weight: 57 kDa.

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.

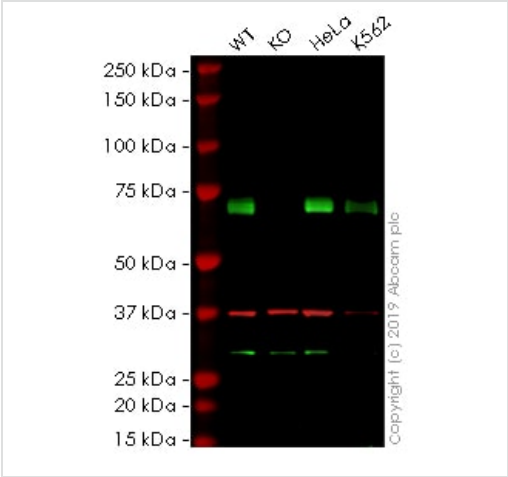
Sequence similarities Belongs to the HSF family.

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.

Cellular localization Cytoplasm. Nucleus. Cytoplasmic during normal growth. On activation, translocates to nuclear stress granules. Colocalizes with SUMO1 in nuclear stress granules.

Images



Western blot - Anti-HSF1 antibody [10H8] (ab61382)

All lanes : Anti-HSF1 antibody [10H8] (ab61382) at 1/1000 dilution

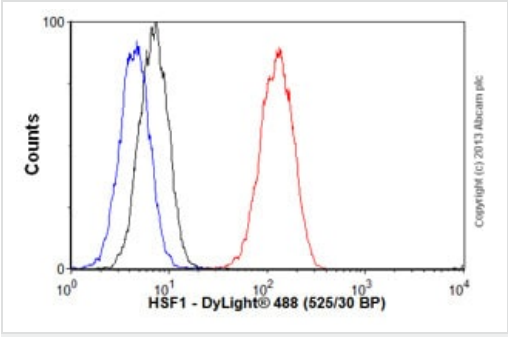
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : Hsf1 knockout HAP1 whole cell lysate
Lane 3 : HeLa whole cell lysate
Lane 4 : K562 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 57 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab61382 observed at 57 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

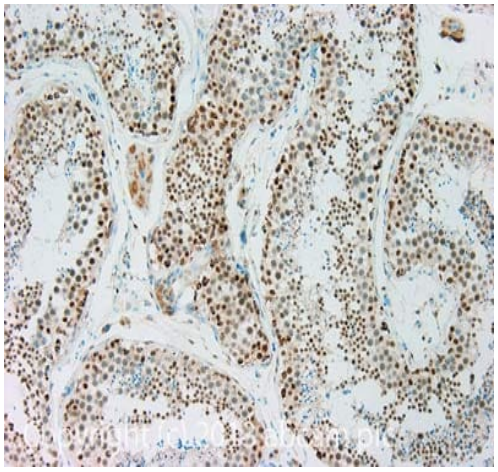
ab61382 was shown to recognize in wild-type HAP1 cells as signal was lost at the expected MW in Hsf1 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and Hsf1 knockout samples were subjected to SDS-PAGE. Ab61382 and **ab181602** (Rabbit anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rat IgG H&L (IRDye® 800CW) preabsorbed and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed **ab216777** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Flow Cytometry - Anti-HSF1 antibody [10H8] (ab61382)

Overlay histogram showing HeLa cells stained with ab61382 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab61382, 1µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-rat IgG (H+L) (**ab98386**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was rat IgG1 [RTK2071] (**ab18412**, 1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line). Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody

gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HSF1 antibody [10H8] (ab61382)

IHC image of HSF1 staining in Human normal testis formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol B. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab61382, 5µg/ml, for 15 mins at room temperature. A Goat anti-Rat biotinylated secondary antibody was used to detect the primary, and visualized using an HRP conjugated ABC system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

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