abcam

Product datasheet

Anti-HSF1 antibody [EP1710Y] - ChIP Grade ab52757

KO VALIDATED Recombinant RabMAb

26 References 15 Images

Overview

Product name	Anti-HSF1 antibody [EP1710Y] - ChIP Grade
Description	Rabbit monoclonal [EP1710Y] to HSF1 - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ChIP, WB, IP, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide within Human HSF1 aa 450 to the C-terminus (C terminal). The exact sequence is proprietary.
Positive control	WB: K562 HAP1 and HeLa whole cell lysate (**ab150035**). ICC/IF: MCF-7 cells. Flow Cyt (intra): HeLa cells. IHC-P: Human ovarian carcinoma tissue; Mouse testis and colon tissue.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

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 long term. Avoid freeze / thaw cycle. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.21% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EP1710Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab52757 in the following tested applications.

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

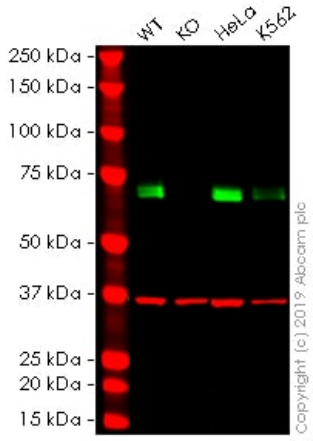
Application	Abreviews	Notes
Flow Cyt (Intra)		1/20. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ChIP		Use at an assay dependent concentration.
WB		1/50000. Detects a band of approximately 85 kDa (predicted molecular weight: 57 kDa). **For unpurified use at 1/100000.**
IP		1/20. **For unpurified use at 1/100.**
IHC-P		1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/100 - 1/250.

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 [EP1710Y] - ChIP Grade (ab52757)

All lanes : Anti-HSF1 antibody [EP1710Y] - ChIP Grade (ab52757) at 1/100000 dilution

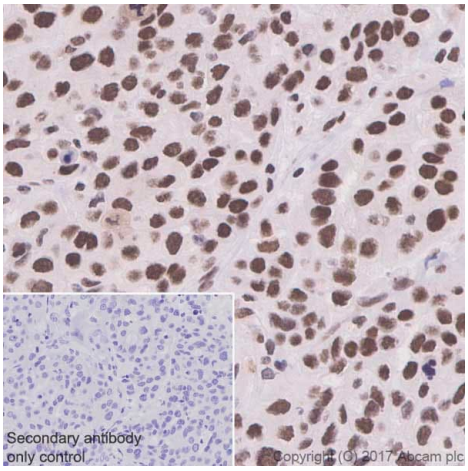
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : Hsf1 knockout HAP1 whole cell lysate
Lane 3 : HeLa whole cell lysate
Lane 4 : K562 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 57 kDa
Observed band size: 57 kDa

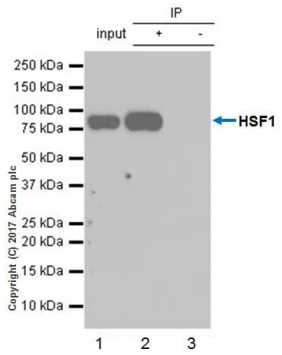
Lanes 1 - 4: Merged signal (red and green). Green - ab52757 observed at 57 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab52757 was shown to specifically react with in wild-type HAP1 cells as signal was lost in Hsf1 knockout cells. Wild-type and Hsf1 knockout samples were subjected to SDS-PAGE. Ab52757 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/100000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HSF1 antibody [EP1710Y] - ChIP Grade (ab52757)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human ovarian carcinoma tissue sections labeling HSF1 with Purified ab52757 at 1:250 dilution (1.06 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.

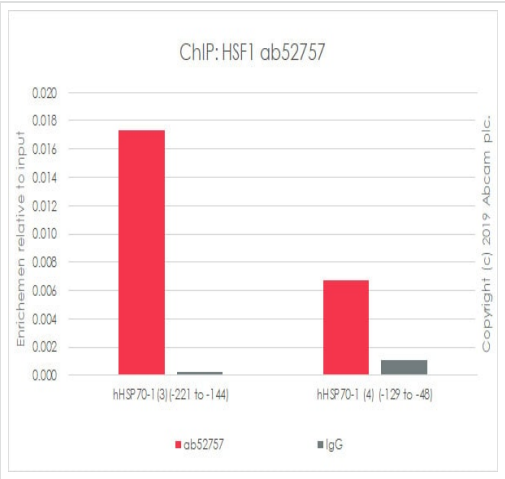


Immunoprecipitation - Anti-HSF1 antibody [EP1710Y] - ChIP Grade (ab52757)

ab52757 (purified) at 1:20 dilution (2µg) immunoprecipitating HSF1 in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10ug
Lane 2 (+): ab52757 & HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab52727** in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.

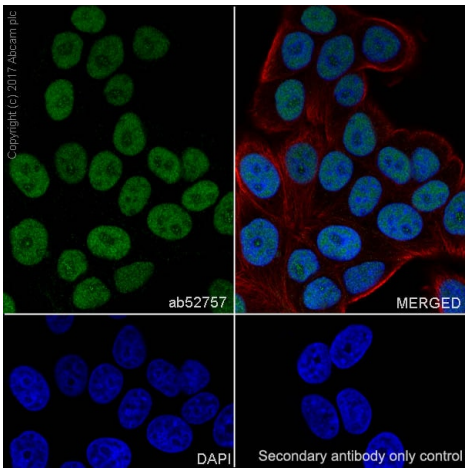


ChIP - Anti-HSF1 antibody [EP1710Y] - ChIP Grade (ab52757)

Chromatin was prepared from HeLa cells heat shocked (42^{o}C 30 minutes) according to the Abcam Dual X-ChIP protocol. Cells were fixed with EGS for 30 minutes, then formaldehyde for 10 minutes. The ChIP was performed with 25 µg of chromatin, 5 µg of ab52757 (red), and 20 µl of Protein A/G sepharose beads. 5 µg of rabbit normal IgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (sybr green approach).

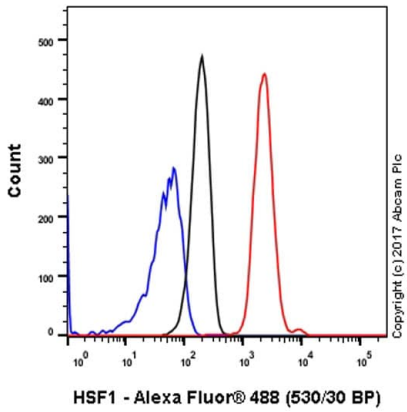
Primers and probes are located in the first kb of the transcribed region.

*http://www.abcam.com/resources?keywords=X%20ChIP%20protocol



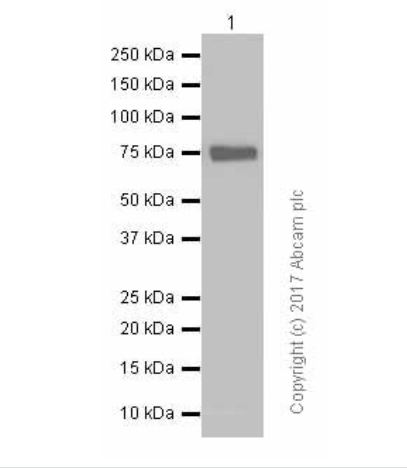
Immunocytochemistry/ Immunofluorescence - Anti-HSF1 antibody [EP1710Y] - ChIP Grade (ab52757)

Immunocytochemistry/ Immunofluorescence analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling HSF1 with Purified ab52757 at 1:100 dilution. Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200. **ab150077** Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Flow Cytometry (Intracellular) - Anti-HSF1 antibody [EP1710Y] - ChIP Grade (ab52757)

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling HSF1 with purified ab52757 at 1/20 dilution (10µg/ml) (red). Cells were fixed with 80% Methanol and permeabilised with 0.1% Tween-20. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



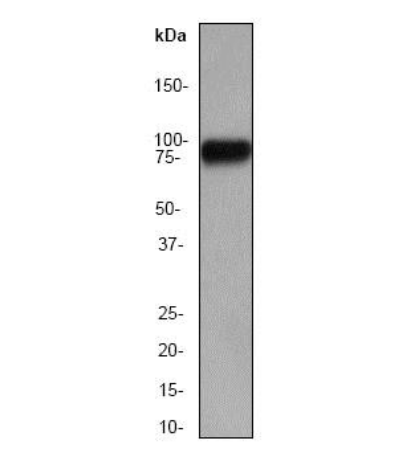
Western blot - Anti-HSF1 antibody [EP1710Y] - ChIP Grade (ab52757)

Anti-HSF1 antibody [EP1710Y] - ChIP Grade (ab52757) at 1/50000 dilution (purified) + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 15 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 57 kDa
Observed band size: 80 kDa

Blocking and diluting buffer: 5% NFDM/TBST

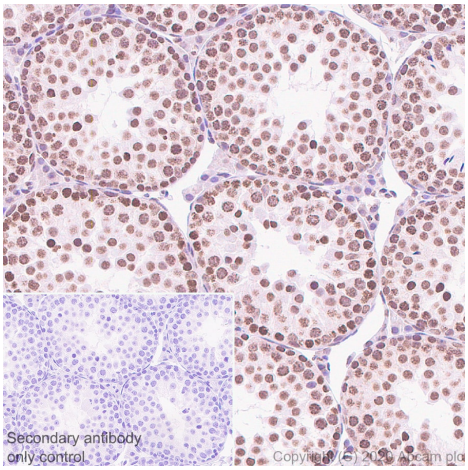


Western blot - Anti-HSF1 antibody [EP1710Y] - ChIP Grade (ab52757)

Anti-HSF1 antibody [EP1710Y] - ChIP Grade (ab52757) at 1/100000 dilution (unpurified) + HeLa cell lysate at 10 µg

Secondary
HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution

Predicted band size: 57 kDa
Observed band size: 85 kDa

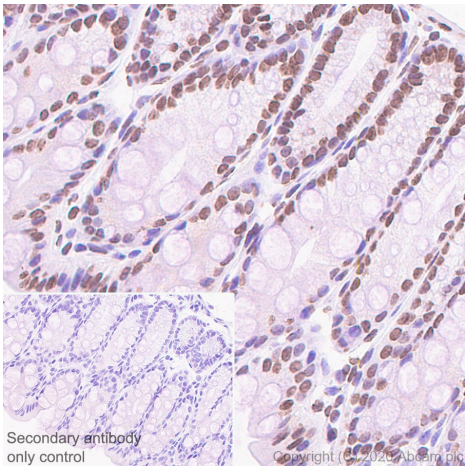


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HSF1 antibody [EP1710Y] - ChIP Grade (ab52757)

Immunohistochemical analysis of paraffin-embedded Mouse testis tissue labeling HSF1 with ab52757 at 1/1000 followed by a Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) Ready to use. Nuclear staining on mouse testis. The section was incubated with ab52757 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**), Ready to use.

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins

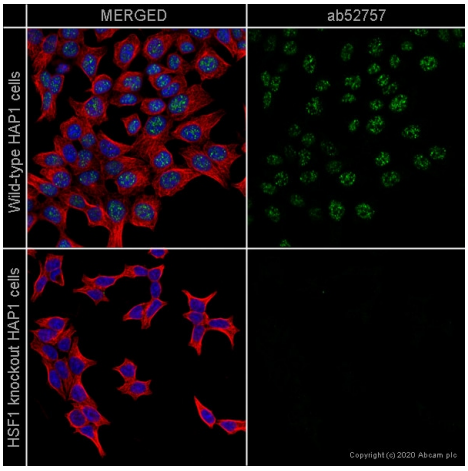


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HSF1 antibody [EP1710Y] - ChIP Grade (ab52757)

Immunohistochemical analysis of paraffin-embedded Mouse colon tissue labeling HSF1 with ab52757 at 1/1000 followed by a Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) Ready to use. Nuclear staining on mouse colon. The section was incubated with ab52757 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**), Ready to use.

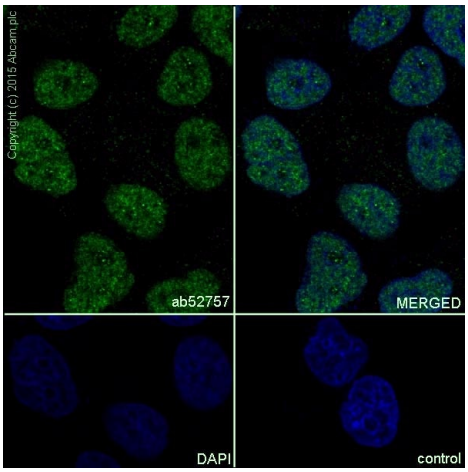
Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins



Immunocytochemistry/ Immunofluorescence - Anti-HSF1 antibody [EP1710Y] - ChIP Grade (ab52757)

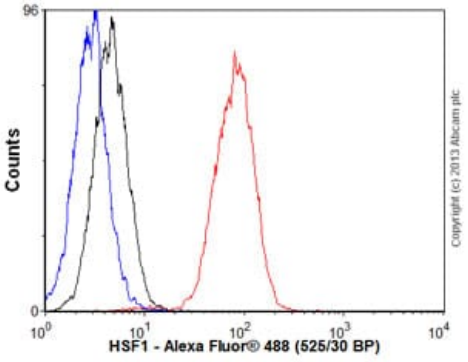
ab52757 staining HSF1 in wild-type Hap1 cells (top panel) and HSF1 knockout Hap1 cells (bottom panel). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab52757 at 1/250 dilution and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-HSF1 antibody [EP1710Y] - ChIP Grade (ab52757)

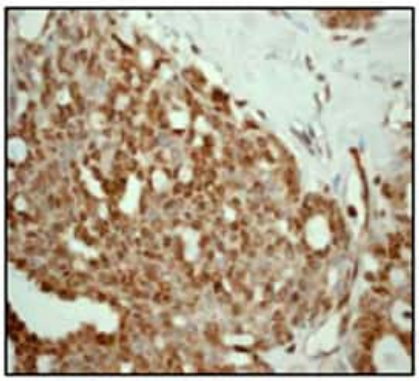
Immunocytochemistry/Immunofluorescence analysis of MCF-7 cells labelling HSF1 with unpurified ab52757 at 1/1000. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody.
Control: PBS only.
Nuclear counter stain: DAPI.



Flow Cytometry (Intracellular) - Anti-HSF1 antibody [EP1710Y] - ChIP Grade (ab52757)

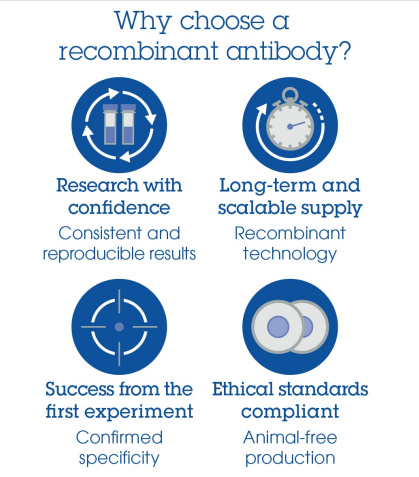
Overlay histogram showing HeLa cells stained withunpurified ab52757 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (unpurified ab52757, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluorr® 488 goat anti-rabbit IgG (H+L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. 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 [EP1710Y] - ChIP Grade (ab52757)

Immunohistochemical staining of paraffin-embedded human ovarian carcinoma using unpurified ab52757 at a 1:100 dilution.



Anti-HSF1 antibody [EP1710Y] - ChIP Grade (ab52757)

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