abcam

Product datasheet

Anti-Hsp27 antibody ab5579

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Overview

Product name	Anti-Hsp27 antibody
Description	Rabbit polyclonal to Hsp27
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human, African green monkey
Immunogen	Synthetic peptide corresponding to Human Hsp27 aa 10-21. Sequence: LLRGPSWDPFRC (Peptide available as **ab39789**) Run BLAST with Run BLAST with
Positive control	WB: HEK-293T, HeLa, K562, A431, HepG2, COS-7, NIH/3T3, MCF7, MDA-MB-231, PC3, DU 145, LNCaP, HT1080 whole cell lysate. IHC-P: Human skeletal muscle and breast carcinoma tissue. ICC/IF: HeLa, MCF-7 and C6 cells.
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 long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.05% Sodium azide Constituents: 99% PBS, 0.1% BSA
Purity	Immunogen affinity purified
Purification notes	Antigen affinity chromatography.

Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab5579 in the following tested applications.

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

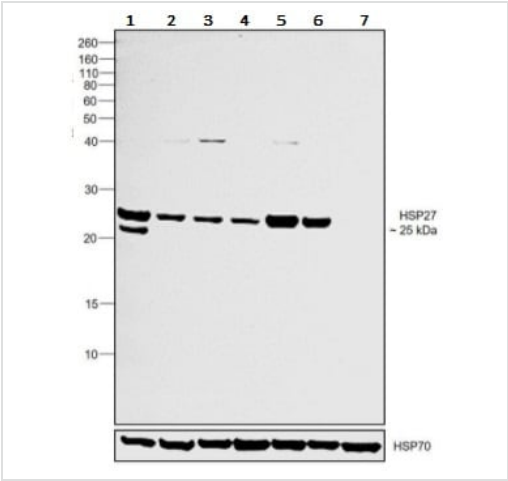
Application	Abreviews	Notes
WB	(1)	1/1000 - 1/2000. Detects a band of approximately 27 kDa.
ICC/IF		1/50.
IHC-P		Use a concentration of 4 µg/ml. Perform heat mediated antigen retrieval via the microwave method before commencing with IHC staining protocol.

Target

Function	Involved in stress resistance and actin organization.
Tissue specificity	Detected in all tissues tested: skeletal muscle, heart, aorta, large intestine, small intestine, stomach, esophagus, bladder, adrenal gland, thyroid, pancreas, testis, adipose tissue, kidney, liver, spleen, cerebral cortex, blood serum and cerebrospinal fluid. Highest levels are found in the heart and in tissues composed of striated and smooth muscle.
Involvement in disease	Defects in HSPB1 are the cause of Charcot-Marie-Tooth disease type 2F (CMT2F) [MIM:606595]. CMT2F is a form of Charcot-Marie-Tooth disease, the most common inherited disorder of the peripheral nervous system. Charcot-Marie-Tooth disease is classified in two main groups on the basis of electrophysiologic properties and histopathology: primary peripheral demyelinating neuropathy or CMT1, and primary peripheral axonal neuropathy or CMT2. Neuropathies of the CMT2 group are characterized by signs of axonal regeneration in the absence of obvious myelin alterations, normal or slightly reduced nerve conduction velocities, and progressive distal muscle weakness and atrophy. Nerve conduction velocities are normal or slightly reduced. CMT2F onset is between 15 and 25 years with muscle weakness and atrophy usually beginning in feet and legs (peroneal distribution). Upper limb involvement occurs later. CMT2F inheritance is autosomal dominant. Defects in HSPB1 are a cause of distal hereditary motor neuronopathy type 2B (HMN2B) [MIM:608634]. Distal hereditary motor neuronopathies constitute a heterogeneous group of neuromuscular disorders caused by selective impairment of motor neurons in the anterior horn of the spinal cord, without sensory deficit in the posterior horn. The overall clinical picture consists of a classical distal muscular atrophy syndrome in the legs without clinical sensory loss. The disease starts with weakness and wasting of distal muscles of the anterior tibial and peroneal compartments of the legs. Later on, weakness and atrophy may expand to the proximal muscles of the lower limbs and/or to the distal upper limbs.
Sequence similarities	Belongs to the small heat shock protein (HSP20) family.
Post-translational modifications	Phosphorylated in MCF-7 cells on exposure to protein kinase C activators and heat shock.
Cellular localization	Cytoplasm. Nucleus. Cytoplasm > cytoskeleton > spindle. Cytoplasmic in interphase cells.

Colocalizes with mitotic spindles in mitotic cells. Translocates to the nucleus during heat shock and resides in sub-nuclear structures known as SC35 speckles or nuclear splicing speckles.

Images



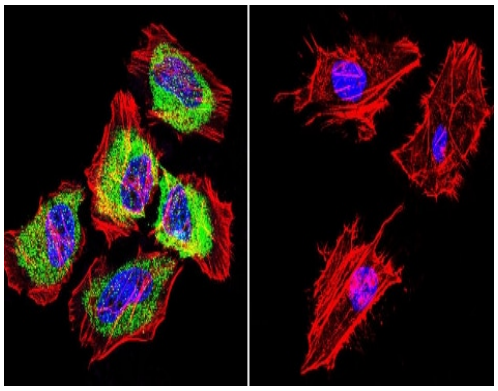
Western blot - Anti-Hsp27 antibody (ab5579)

All lanes : Anti-Hsp27 antibody (ab5579) at 1/2000 dilution

Lane 1 : MCF7 (human breast adenocarcinoma cell line) whole cell lysate
Lane 2 : MDA-MB-231 (human breast adenocarcinoma cell line) whole cell lysate
Lane 3 : PC3 (human prostate adenocarcinoma cell line) whole cell lysate
Lane 4 : DU 145 (human prostate carcinoma cell line) whole cell lysate
Lane 5 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 6 : LNCaP (human prostate cancer cell line) whole cell lysate
Lane 7 : HT1080 (human fibrosarcoma cell line) whole cell lysate

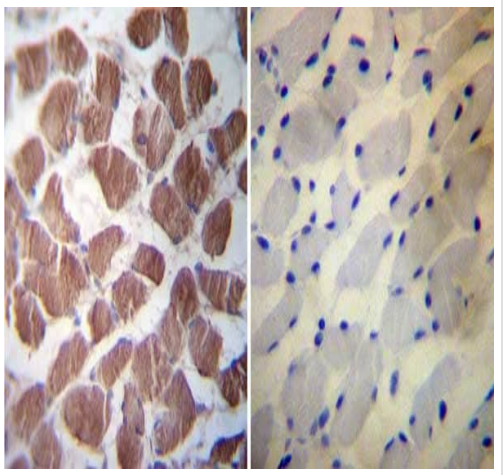
Lysates/proteins at 30 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG (H+L) Superclonal™ Recombinant Secondary Antibody, HRP at 1/4000 dilution



Immunocytochemistry/ Immunofluorescence - Anti-Hsp27 antibody (ab5579)

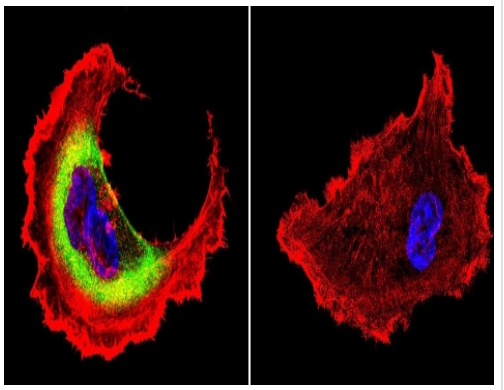
Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Hsp27 (green) with ab5579 at 1/200 dilution, followed by DyLight 488-conjugated secondary antibody. F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue). Cells were fixed with formaldehyde and incubated with the primary antibody overnight at 4°C. 60X magnification. Right - negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp27 antibody (ab5579)

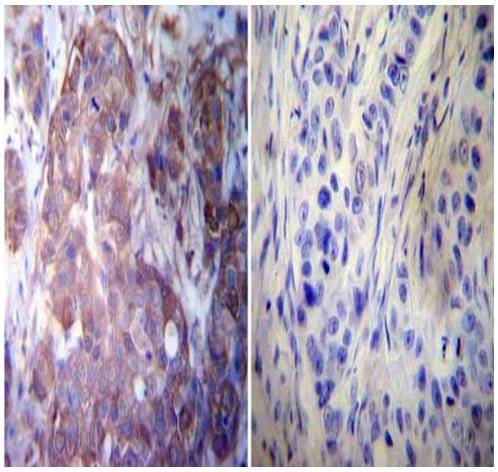
Immunohistochemical analysis of both normal and cancer biopsies of deparaffinized human skeletal muscle tissue labeling Hsp27 with ab5579 at 1/20 dilution or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP, followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.

To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH 6.0) buffer, microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp27 antibody (ab5579)

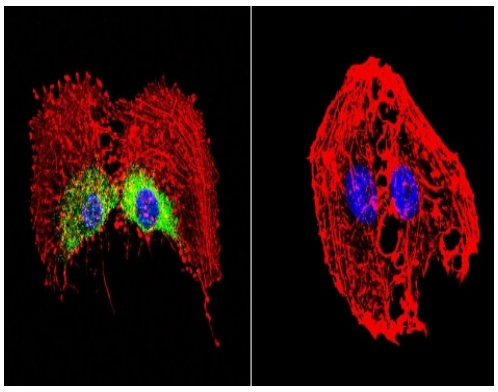
Immunofluorescence analysis of MCF7 (Human breast adenocarcinoma cell line) cells labeling Hsp27 (green) with ab5579 at 1/200 dilution, followed by DyLight 488-conjugated secondary antibody. F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue). Cells were fixed with formaldehyde and incubated with the primary antibody overnight at 4°C. 60X magnification. Right - negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp27 antibody (ab5579)

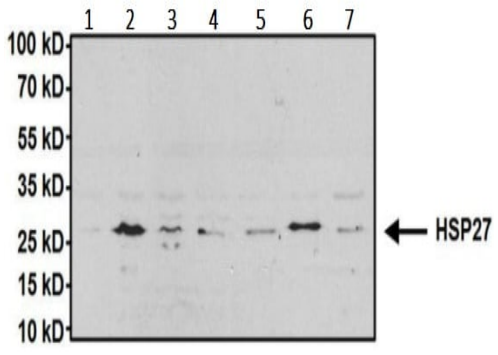
Immunohistochemical analysis of both normal and cancer biopsies of deparaffinized human breast carcinoma tissue labeling Hsp27 with ab5579 at 1/100 dilution or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP, followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.

To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer, microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp27 antibody (ab5579)

Immunofluorescence analysis of C6 (Rat glial tumor cell line) cells labeling Hsp27 (green) with ab5579 at 1/100 dilution, followed by DyLight 488-conjugated secondary antibody. F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue). Cells were fixed with formaldehyde and incubated with the primary antibody overnight at 4°C. 60X magnification. Right - negative control.



Western blot - Anti-Hsp27 antibody (ab5579)

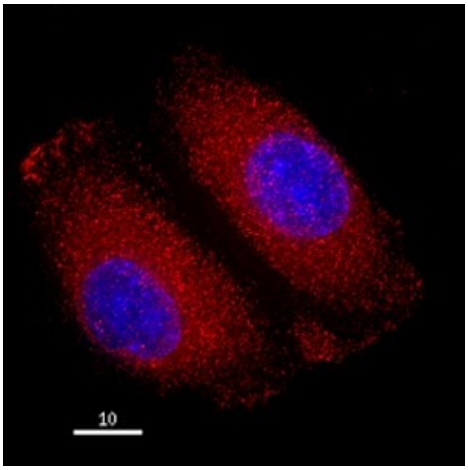
All lanes : Anti-Hsp27 antibody (ab5579) at 1/1000 dilution

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 lymphoblast cell line) 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 embryonic fibroblast cell line) whole cell lysate

Lysates/proteins at 50 µg per lane.

Secondary

All lanes : Goat anti-rabbit IgG-HRP secondary antibody at 1/20000 dilution



Immunocytochemistry/ Immunofluorescence - Anti-Hsp27 antibody (ab5579)

This image is courtesy of Michael Manicini, Ph.D.

HeLa (human epithelial cell line from cervix adenocarcinoma) cells were fixed with 4% formaldehyde in PEM buffer. The coverslip was incubated in blocking buffer of 5% powdered milk in TBS-T plus 0.02% sodium azide for 1 hour at room temperature. Blocking buffer was removed and primary antibody was added at a dilution of 1/250 and incubated overnight at 4 degrees celsius. The coverslips were then washed 4-5 times with blocking buffer for 5 minutes. Secondary antibody, goat anti-rabbit Alexa 594 (**ab150080**), was added at a dilution of 1/1000 and incubated at room temperature for one hour. From this point on coverslips were covered with foil to protect them from light. They were washed 5 times with TBS-T and then one time with PEM, for 5 minutes each wash. The coverslips were fixed 10-30 minutes in 4% formaldehyde in PEM buffer, then washed 3 times with PEM buffer for 5 minutes. 0.1M ammonium chloride in PEM buffer was added for 10 minutes to quench auto-florescence, and then slips were washed 2 times for 5 minutes in PEM followed by 3 washes for 5 minute