abcam

Product datasheet

Anti-Hsp27 (phospho S15) antibody ab5581

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Overview

Product name	Anti-Hsp27 (phospho S15) antibody
Description	Rabbit polyclonal to Hsp27 (phospho S15)
Host species	Rabbit
Specificity	ab5581 detects phosphorylated heat shock protein 27(hsp27) from rat and Human tissues.
Tested applications	**Suitable for:** WB, ICC/IF, IP
Species reactivity	**Reacts with:** Human **Predicted to work with:** Cow, Dog, Pig
Immunogen	Synthetic peptide corresponding to Human Hsp27 aa 10-21 (phospho S15). Sequence: LLRGPSWDPFRC (Peptide available as **ab41772**) Run BLAST with Run BLAST with
Positive control	ICC/IF: HeLa cells. WB: HeLa cell lysate. IP: HeLa 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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.05% Sodium azide Constituents: 0.1% BSA, 99% PBS
Purity	Immunogen affinity purified

Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab5581 in the following tested applications.

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

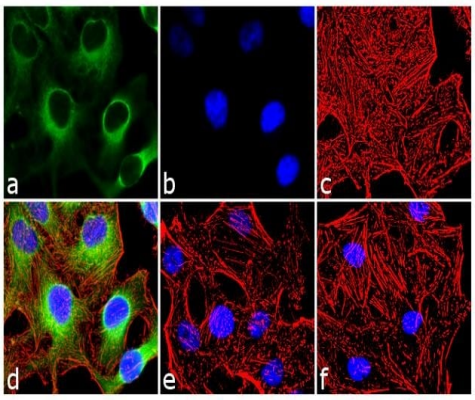
Application	Abreviews	Notes
WB		1/100 - 1/1000. Predicted molecular weight: 23 kDa.
ICC/IF		1/50 - 1/200.
IP		Use at an assay dependent concentration. 3 µg

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

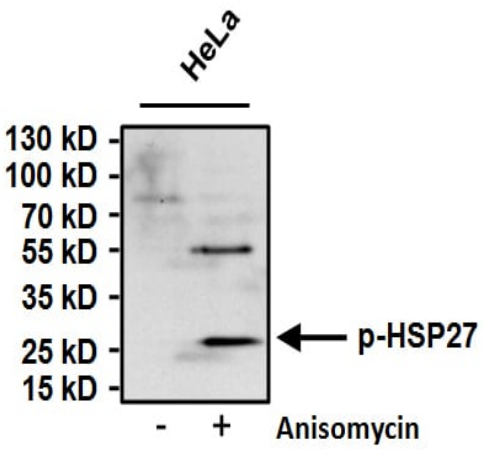
and resides in sub-nuclear structures known as SC35 speckles or nuclear splicing speckles.

Images



Immunocytochemistry/ Immunofluorescence - Anti-Hsp27 (phospho S15) antibody (ab5581)

Immunocytochemistry analysis of Hsp27 using ab5581 at 2µg/mL concentration shows staining in 4% paraformaldehyde-fixed 0.1% Triton X-100 permeabilized HeLa Cells treated with Anisomysin. The secondary antibody was Goat anti-Rabbit IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate. Hsp27 (green), F-Actin staining with Alexa Fluor® 555 Rhodamine Phalloidin (red) and nuclei with SlowFade® Gold Antifade Mountant with DAPI (blue) is shown. Negative control has no primary antibody.



Western blot - Anti-Hsp27 (phospho S15) antibody (ab5581)

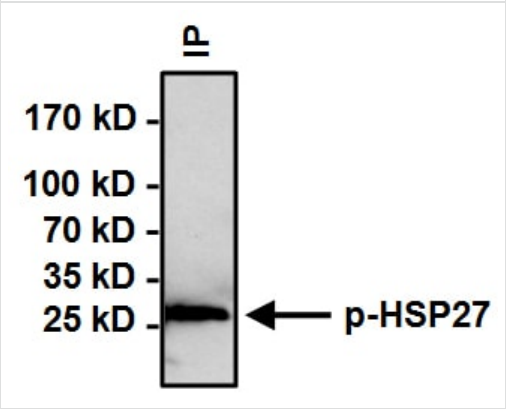
All lanes : Anti-Hsp27 (phospho S15) antibody (ab5581) at 1/500 dilution

Lane 1 : HeLa cell lysate - untreated
Lane 2 : HeLa cell lysate - treated with 10nM Anisomycin

Lysates/proteins at 50 µg per lane.

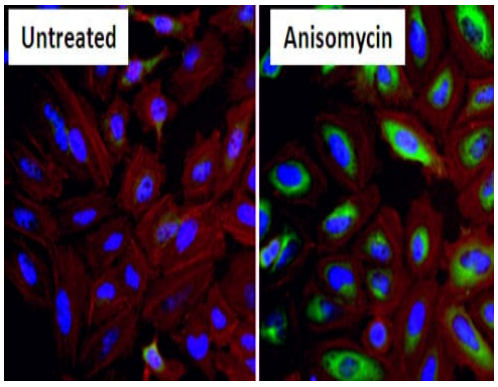
Secondary
All lanes : HRP-conjugated goat anti-rabbit IgG at 1/20000 dilution

Predicted band size: 23 kDa



Immunoprecipitation - Anti-Hsp27 (phospho S15) antibody (ab5581)

Immunoprecipitation of Hsp27 (phospho S15) was performed on HeLa cells treated with 10uM Anisomysin for 30 minutes. Antigen-antibody complexes were formed by incubating 500ug of whole cell lysate with 3ug of ab5581 overnight on a rocking platform at 4°C. The immune complexes were captured on 50ul Protein A/G Agarose, washed extensively, and eluted with Lane Marker Reducing Sample Buffer. Samples were then 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 probed with ab5581 at 1:500 overnight rotating at 4°C, washed with TBST, and probed with Clean-Blot IP Detection Reagent at 1/1000 for at least 1 hour. Chemiluminescent detection was performed using SuperSignal West Dura.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp27 (phospho S15) antibody (ab5581)

Immunocytochemistry/Immunofluorescence analysis of Hsp27 (phospho S15) (green) in HeLa cells either untreated (left) or treated with 10uM Anisomysin (right) for 30 minutes. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% blocker BSA for 15 minutes at room temperature. Cells were incubated with ab5581 at 1:50 for at least 1 hour at room temperature, washed with PBS, and incubated with DyLight 488 goat anti-rabbit IgG secondary antibody (1:400) for 30 minutes at room temperature. F-Actin (red) was stained with DyLight 554 Phalloidin and nuclei (blue) were stained with Hoechst 33342 dye. 20X magnification.

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