abcam

Product datasheet

Anti-Hsp27 (phospho S78) antibody [Y175] ab32501

Recombinant RabMAb

8 References 5 Images

Overview

Product name	Anti-Hsp27 (phospho S78) antibody [Y175]
Description	Rabbit monoclonal [Y175] to Hsp27 (phospho S78)
Host species	Rabbit
Specificity	ab32501 recognises Hsp27 (phospho S78). The antibody will detect Src phosphorylation on Serine 78. This antibody does not react with mouse and rat species in the Western blot application.
Tested applications	**Suitable for:** ICC/IF, WB, IHC-P, Dot blot **Unsuitable for:** Flow Cyt
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa starved overnight then treated with 250 ng/ml anisomycin for 30 minutes whole cell lysate. IHH: Human breast cancer tissue sections. ICC/IF: HeLa treated with 25 ug/mL anisomycin for 30 min, then Lambda Protein Phosphatase 31 for 2 hours cells.
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. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y175
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32501 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
ICC/IF		1/50.
WB		1/5000. Predicted molecular weight: 23 kDa.
IHC-P		1/5000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Dot blot		1/2000.

Application notes Is unsuitable for Flow Cyt.

Target

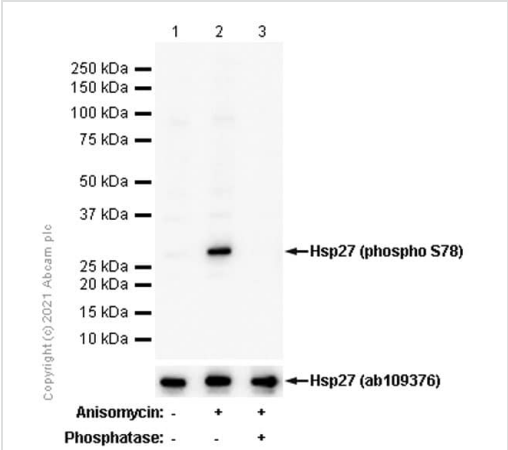
Function Involved in stress resistance and actin organization.

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 (phospho S78) antibody [Y175] (ab32501)

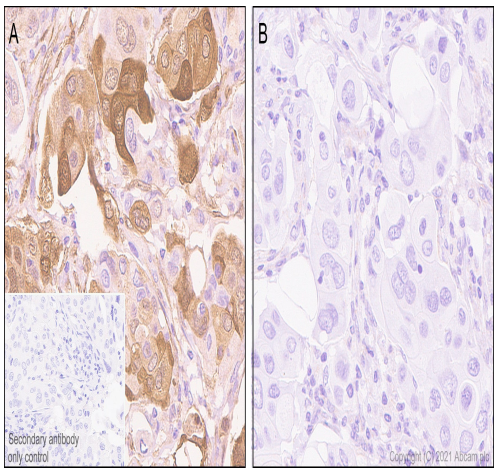
All lanes : Anti-Hsp27 (phospho S78) antibody [Y175] (ab32501) at 1/5000 dilution (Purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) starved overnight then treated with 250 ng/ml anisomycin for 30 minutes whole cell lysate
Lane 3 : HeLa (Human cervix adenocarcinoma epithelial cell) starved overnight then treated with 250 ng/ml anisomycin for 30 minutes whole cell lysate, and then the menbrane treated with Alkaline Phosphatase for 1 hour

Lysates/proteins at 15 µg per lane.

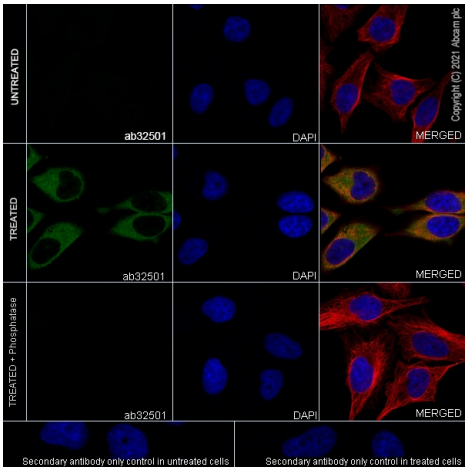
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 23 kDa
Observed band size: 27 kDa



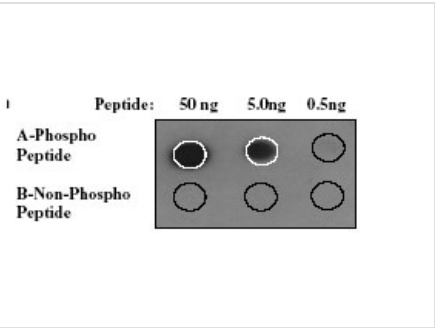
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp27 (phospho S78) antibody [Y175] (ab32501)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast cancer tissue sections labelling Hsp27 with purified ab32501 at 1/5000 dilution (0.11 µg/ml). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used at 1/0 dilution. PBS instead of the primary antibody was used as the negative control. Positive staining on human breast cancer without alkaline phosphatase treatment (image A). No staining on human breast cancer with alkaline phosphatase treatment (image B). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



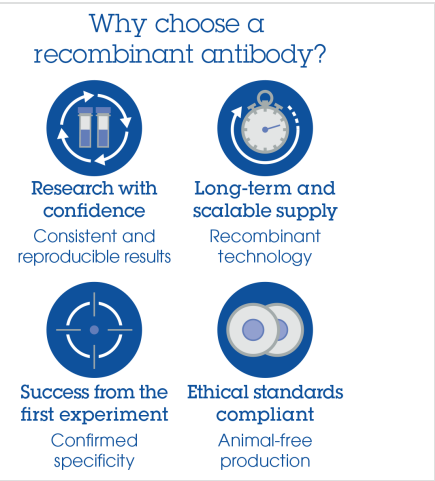
Immunocytochemistry/ Immunofluorescence - Anti-Hsp27 (phospho S78) antibody [Y175] (ab32501)

Immunocytochemistry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) treated with 25 ug/mL anisomycin for 30 min, then Lambda Protein Phosphatase 31? for 2 hours cells labeling Hsp27 with purified ab32501 at 1/50 dilution (11.3 µg/mL). 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 (2.5 µg/mL). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 µg/mL) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Dot Blot - Anti-Hsp27 (phospho S78) antibody [Y175] (ab32501)

Dot Blot analysis on immunogen phospho-peptide (A) and non-phospho peptide (B) using ab32501 at dilution 1/2000.



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