


Product datasheet

Anti-Hsp27 antibody [EPR5477] ab109376

KO **VALIDATED** Recombinant RabMAb

★★★★★ [2 Abreviews](#) [7 References](#) [6 Images](#)

Overview

Product name	Anti-Hsp27 antibody [EPR5477]
Description	Rabbit monoclonal [EPR5477] to Hsp27
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), ICC/IF, WB
Species reactivity	Reacts with: Human, African green monkey Predicted to work with: Rat 
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, HAP1, MCF7, COS-1, BxPC-3 and HT-1376 cell lysates. ICC/IF: HeLa cells. Flow Cyt (intra): HAP1 cells.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	<p>pH: 7.20</p> <p>Preservative: 0.05% Sodium azide</p> <p>Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue culture supernatant</p>
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR5477

Isotype

IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab109376 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)		1/10 - 1/100. ab172730 -Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/500.
WB	★★★★★ (2)	1/1000 - 1/10000. Predicted molecular weight: 23 kDa.

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

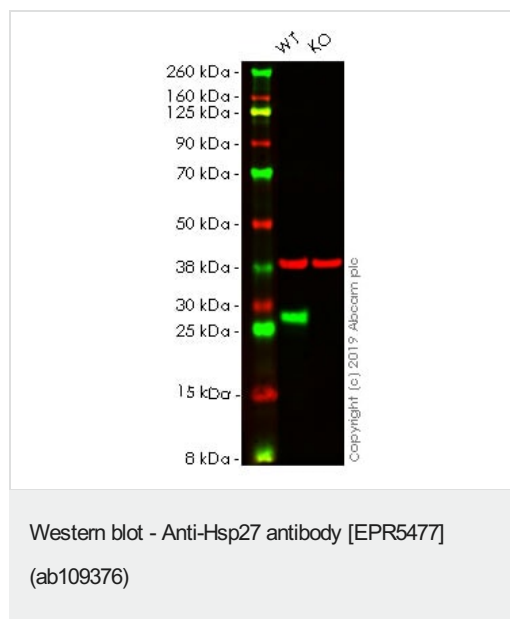
Phosphorylated in MCF-7 cells on exposure to protein kinase C activators and heat shock.

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



All lanes : Anti-Hsp27 antibody [EPR5477] (ab109376) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : HSPB1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

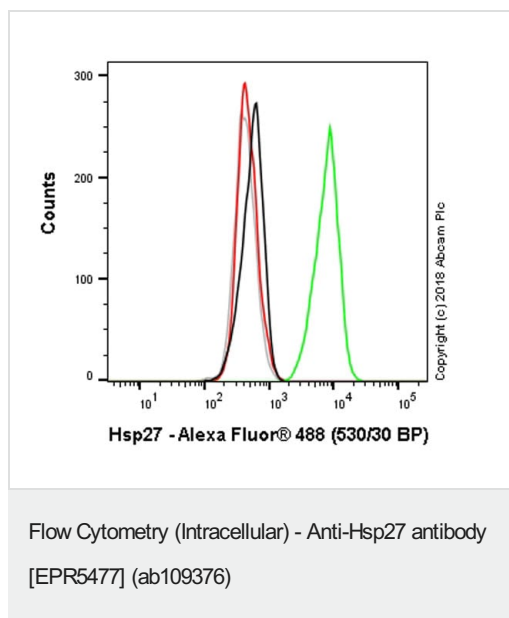
Performed under reducing conditions.

Predicted band size: 23 kDa

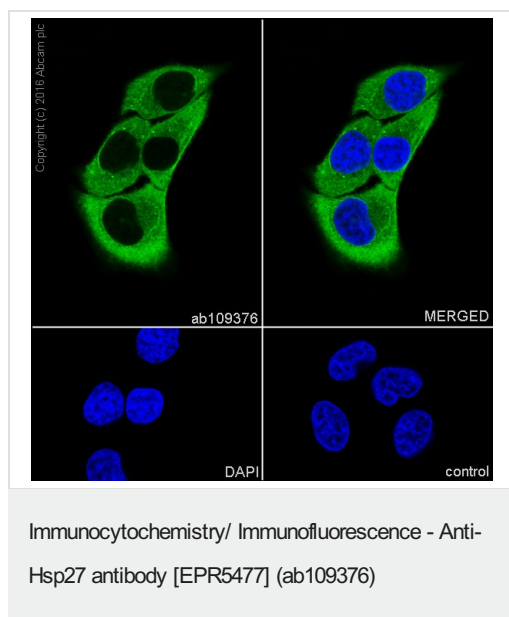
Observed band size: 23 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab109376 observed at 23 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) observed at 37 kDa.

ab109376 was shown to react with Hsp27 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line [ab261738](#) (knockout cell lysate [ab256945](#)) was used. Wild-type HeLa and HSPB1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab109376 and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed ([ab216776](#)) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

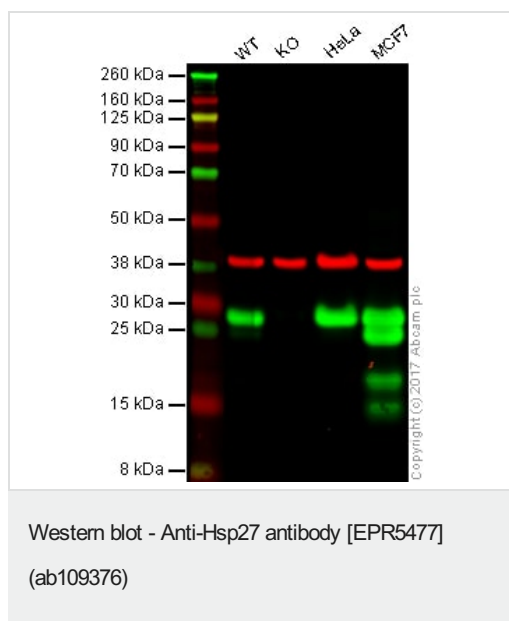


Overlay histogram showing wild-type HAP1 (green line) and HSPB1 knockout HAP1 cells (red line) stained with ab109376. The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab109376, 1 µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) presorbed (**ab150081**) at 1/2000 dilution for 30 min at 22°C. A rabbit IgG isotype control antibody (**ab172730**) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, knockout HSPB1 HAP1- grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.



Immunocytochemistry/Immunofluorescence analysis of HeLa (human cervix adenocarcinoma) cells labelling Hsp27 with purified ab109376 at 1/500. Cells were fixed with 100% methanol. **ab150077**, Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. Nuclei were counterstained with DAPI (blue).

Secondary Only Control: PBS was used instead of the primary antibody as the negative control.



All lanes : Anti-Hsp27 antibody [EPR5477] (ab109376) at 1/1000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate

Lane 2 : Hsp27 knockout HAP1 whole cell lysate

Lane 3 : HeLa whole cell lysate

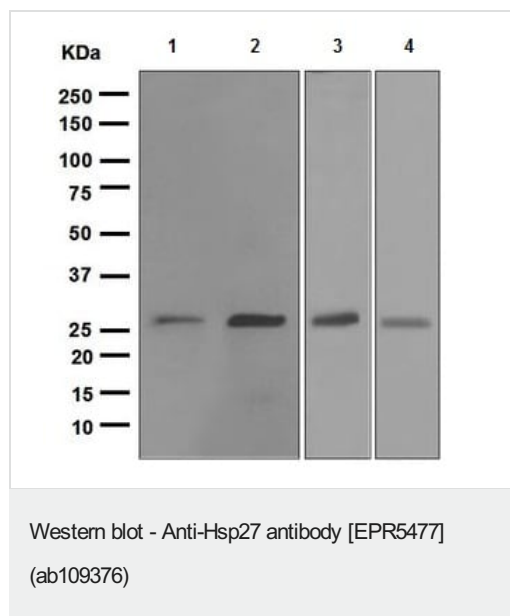
Lane 4 : MCF7 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 23 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab109376 observed at 27 kDa. Red - loading control, [ab8245](#), observed at 37 kDa.

ab109376 was shown to specifically react with Hsp27 in wild-type HAP1 cells as signal was lost in Hsp27 knockout HAP1 cells. Wild-type and Hsp27 knockout samples were subjected to SDS-PAGE. Ab109376 and [ab8245](#) (Mouse 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-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.



All lanes : Anti-Hsp27 antibody [EPR5477] (ab109376) at 1/1000 dilution

Lane 1 : HeLa cell lysate

Lane 2 : COS-1 cell lysate

Lane 3 : BxPC-3 cell lysate

Lane 4 : HT-1376 cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 23 kDa

Observed band size: 27 kDa

Why choose a recombinant antibody?

Research with confidence
Consistent and reproducible results

Long-term and scalable supply
Recombinant technology

Success from the first experiment
Confirmed specificity

Ethical standards compliant
Animal-free production

Anti-Hsp27 antibody [EPR5477] (ab109376)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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