abcam

Product datasheet

HRP Anti-Hsp27 antibody [EPR5477] ab194079

KO VALIDATED Recombinant RabMAb

4 Images

Overview

Product name	HRP Anti-Hsp27 antibody [EPR5477]
Description	HRP Rabbit monoclonal [EPR5477] to Hsp27
Host species	Rabbit
Conjugation	HRP
Tested applications	**Suitable for:** IHC-P, WB
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa cell lysate. IHC: normal human skeletal muscle 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.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at -20°C. Avoid freeze / thaw cycle. Store In the Dark.
Storage buffer	pH: 7.40 Preservative: 0.1% Proclin 300 Solution Constituents: 30% Glycerol (glycerin, glycerine), 1% BSA, PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR5477

Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab194079 in the following tested applications.

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

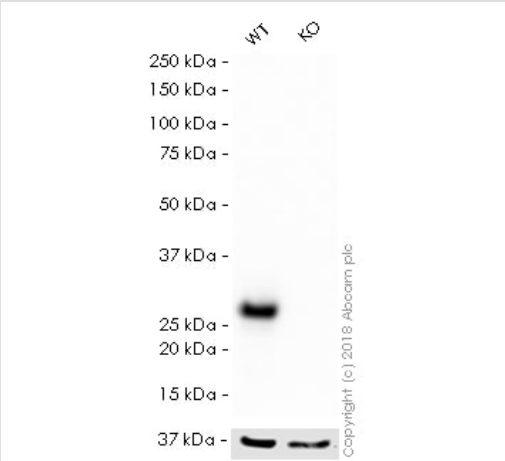
Application	Abreviews	Notes
IHC-P		1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. ab199507 - Rabbit monoclonal IgG (HRP), is suitable for use an as isotype control with this antibody.
WB		1/5000. Detects a band of approximately 27 kDa (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



Western blot - HRP Anti-Hsp27 antibody [EPR5477] (ab194079)

All lanes : HRP Anti-Hsp27 antibody [EPR5477] (ab194079) at 1/5000 dilution

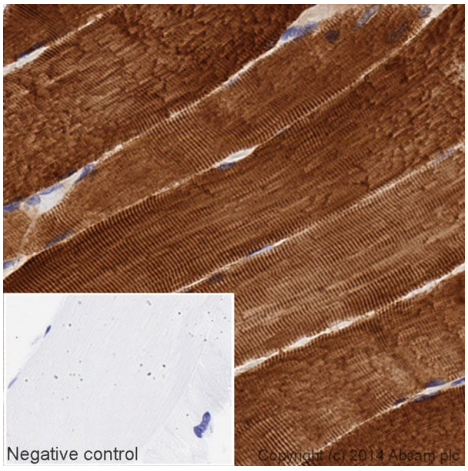
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : HSPB1 (Hsp27) knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Exposure time: 10 seconds

ab194079 was shown to specifically react with Hsp27 in wild-type HAP1 cells as signal was lost in HSPB1 (Hsp27) knockout cells. Wild-type and HSPB1 (Hsp27) knockout samples were subjected to SDS-PAGE. Ab194079 and ab184095 (Mouse monoclonal [mAbcam 9484] to GAPDH - Loading Control (Alexa Fluor® 680) loading control) were incubated overnight at 4°C at 1/5000 dilution and 1/1000 dilution respectively. The loading control was imaged using the Licor Odyssey CLx prior to blots being developed with ECL technique.

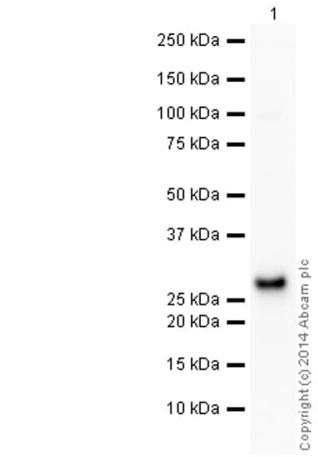


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - HRP Anti-Hsp27 antibody [EPR5477] (ab194079)

IHC image of Hsp27 staining in a section of formalin-fixed paraffin-embedded normal human skeletal muscle tissue*, performed on a Leica BOND. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab194079 at 1/500 dilution, for 15 mins at room temperature. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset negative control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Western blot - HRP Anti-Hsp27 antibody [EPR5477] (ab194079)

HRP Anti-Hsp27 antibody [EPR5477] (ab194079) at 1/5000 dilution + HeLa whole cell lysate (ab150035) at 10 µg

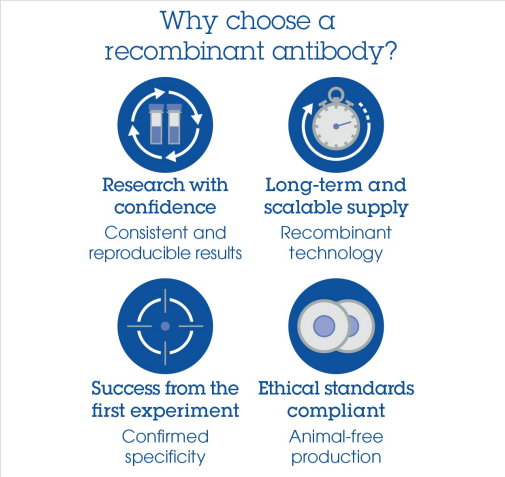
Developed using the ECL technique.

Performed under reducing conditions.

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

Exposure time: 20 seconds

This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab194079 overnight at 4°C. Antibody binding was visualised using ECL development solution ab133406.



HRP Anti-Hsp27 antibody [EPR5477] (ab194079)

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