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Product datasheet

Anti-Tropomyosin 3 antibody [3D5AH3AB4] ab113692

KO VALIDATED

1 Abreviews 4 References 5 Images

Overview

Product name	Anti-Tropomyosin 3 antibody [3D5AH3AB4]
Description	Mouse monoclonal [3D5AH3AB4] to Tropomyosin 3
Host species	Mouse
Tested applications	**Suitable for:** IHC-P, WB, ICC/IF, Flow Cyt
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Tissue, cells or virus. This information is considered to be commercially sensitive.
Positive control	WB: HEK293T, HDFN, H9C2, H4IIE, HepG2 and MEF cell lysates. Flow Cyt: A431 cells. ICC: HDFn cells. IHC-P: Human heart tissue.
General notes	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. 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 Product was previously marketed under the MitoSciences sub-brand.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C.
Storage buffer	pH: 7.5 Preservative: 0.02% Sodium azide Constituent: HEPES buffered saline
Purity	Proprietary Purification
Purification notes	The antibody was produced in vitro using hybridomas grown in serum-free medium, and then

purified by biochemical fractionation. Purity >95% by SDS-PAGE.

Clonality	Monoclonal
Clone number	3D5AH3AB4
Isotype	IgG2b
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab113692 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)	Use a concentration of 10 µg/ml.
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 32 kDa (predicted molecular weight: 32 kDa).
ICC/IF		Use a concentration of 2 µg/ml.
Flow Cyt		Use 0.1µg for 10^6 cells. **ab170192** - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.

Target

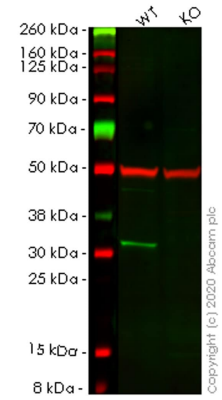
Function Binds to actin filaments in muscle and non-muscle cells. Plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction. Smooth muscle contraction is regulated by interaction with caldesmon. In non-muscle cells is implicated in stabilizing cytoskeleton actin filaments.

Involvement in disease Defects in TPM3 are the cause of nemaline myopathy type 1 (NEM1) [MIM:609284]. A form of nemaline myopathy with autosomal dominant or recessive inheritance. Nemaline myopathies are muscular disorders characterized by muscle weakness of varying severity and onset, and abnormal thread-or rod-like structures in muscle fibers on histologic examination. Autosomal dominant nemaline myopathy type 1 is characterized by a moderate phenotype with onset between birth and early second decade of life. Weakness is diffuse and symmetric with slow progression often with need for a wheelchair in adulthood. The autosomal recessive form has onset at birth with moderate-to-severe hypotonia and diffuse weakness. In the most severe cases, death can occur before 2 years. Less severe cases have delayed major motor milestones, and these patients may walk, but often need a wheelchair before 10 years.
Defects in TPM3 are a cause of thyroid papillary carcinoma (TPC) [MIM:188550]. TPC is a common tumor of the thyroid that typically arises as an irregular, solid or cystic mass from otherwise normal thyroid tissue. Papillary carcinomas are malignant neoplasm characterized by the formation of numerous, irregular, finger-like projections of fibrous stroma that is covered with a surface layer of neoplastic epithelial cells. Note=A chromosomal aberration involving TPM3 is found in thyroid papillary carcinomas. A rearrangement with NTRK1 generates the TRK fusion transcript by fusing the amino end of isoform 2 of TPM3 to the 3'-end of NTRK1.

Sequence similarities Belongs to the tropomyosin family.

Domain	The molecule is in a coiled coil structure that is formed by 2 polypeptide chains. The sequence exhibits a prominent seven-residues periodicity.
Cellular localization	Cytoplasm > cytoskeleton.

Images



Western blot - Anti-Tropomyosin 3 antibody [3D5AH3AB4] (ab113692)

All lanes : Anti-Tropomyosin 3 antibody [3D5AH3AB4] (ab113692) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate
Lane 2 : TPM3 knockout HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

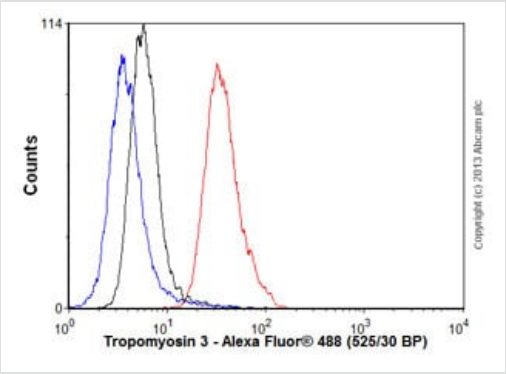
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) at 1/10000 dilution

Predicted band size: 32 kDa
Observed band size: 37 kDa

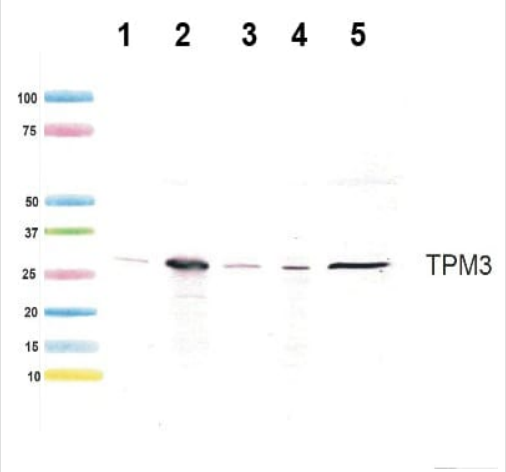
Lanes 1-2: Merged signal (red and green). Green - ab113692 observed at 37 kDa. Red - loading control **ab52901** observed at kDa.

ab113692 Anti-Tropomyosin 3 antibody [3D5AH3AB4] was shown to specifically react with Tropomyosin 3 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line **ab266422** (knockout cell lysate **ab258245**) was used. Wild-type and Tropomyosin 3 knockout samples were subjected to SDS-PAGE. ab113692 and Anti-beta Tubulin [EP1331Y] - Microtubule Marker (**ab52901**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) and Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Flow Cytometry - Anti-Tropomyosin 3 antibody [3D5AH3AB4] (ab113692)

Overlay histogram showing A431 cells stained with ab113692 (red line). The cells were fixed with 80% methanol (5 min)/ and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab113692, 0.1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-mouse IgG (H&L) (**ab150113**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (**ab91366**, 1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Western blot - Anti-Tropomyosin 3 antibody [3D5AH3AB4] (ab113692)

All lanes : Anti-Tropomyosin 3 antibody [3D5AH3AB4] (ab113692) at 1 µg/ml

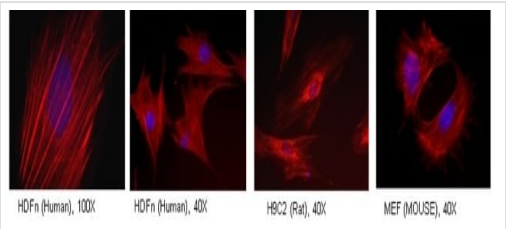
Lane 1 : HepG2 (human)
Lane 2 : HDFN (human)
Lane 3 : H9C2 (rat)
Lane 4 : H4IIE (rat)
Lane 5 : MEF (mouse)

Lysates/proteins at 20 µg per lane.

Developed using the ECL technique.

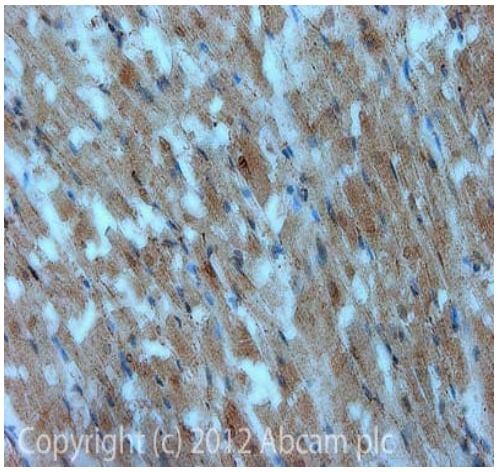
Performed under reducing conditions.

Predicted band size: 32 kDa



Immunocytochemistry/ Immunofluorescence - Anti-Tropomyosin 3 antibody [3D5AH3AB4] (ab113692)

ab113692 stained human fibroblast (HDFn) cells, rat cardiomyocytes (H9C2 cells) and mouse embryo fibroblast (MEF) cells. The cells were paraformaldehyde fixed (4%, 20 min) and Triton X-100 permeabilized (0.1%, 15 min). The cells were incubated with the antibody (3D5AH3AB4,5 µg/ml) for 2h at room temperature or over night at 4°C. The secondary antibody was (red) Alexa Fluor® 594 goat anti-mouse IgG2b (H+L) at a 1/1000 dilution for 1h. 10% Goat serum was used as the blocking agent for all blocking steps. The target protein locates to cytoskeleton.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Tropomyosin 3 antibody [3D5AH3AB4] (ab113692)

IHC image of Tropomyosin 3 staining in Human heart formalin fixed paraffin embedded tissue section, performed on a Leica BondTM system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab113692, 10µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

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.

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