abcam

Product datasheet

Anti-Caldesmon/CDM antibody [E89] ab32330

KO VALIDATED Recombinant RabMAb

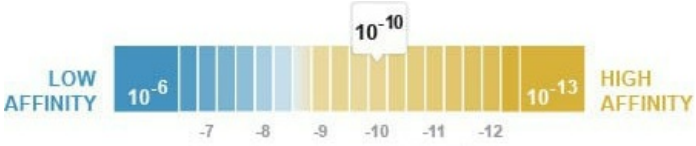
27 References 9 Images

Overview

Product name	Anti-Caldesmon/CDM antibody [E89]
Description	Rabbit monoclonal [E89] to Caldesmon/CDM
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, Flow Cyt (Intra), IP, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa and NIH/3T3 cell lysates; IHC-P: Human breast carcinoma, Human leiomyoma and mouse kidney tissue; ICC/IF: NIH/3T3 cells; IP: NIH/3T3 whole cell lysate; Flow Cyt (Intra): NIH/3T3 cells. WB: HeLa, NIH/3T3 whole cell lysates. Rat Liver lysate.
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). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Dissociation constant (K_D)	$K_D = 1.25 \times 10^{-10}$ M



Learn more about K_D

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	E89
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32330 in the following tested applications.

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

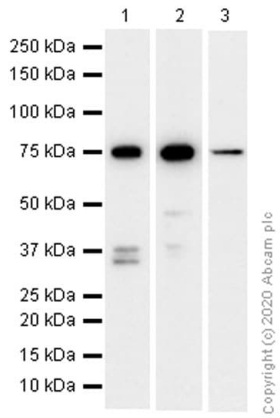
Application	Abreviews	Notes
WB		1/10000 - 1/20000. Detects a band of approximately 70 kDa (predicted molecular weight: 93 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/20. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP		1/20.
ICC/IF		1/50. **For unpurified use at 1/250 - 1/500.**

Target

Function	Actin- and myosin-binding protein implicated in the regulation of actomyosin interactions in smooth muscle and nonmuscle cells (could act as a bridge between myosin and actin filaments). Stimulates actin binding of tropomyosin which increases the stabilization of actin filament structure. In muscle tissues, inhibits the actomyosin ATPase by binding to F-actin. This inhibition is attenuated by calcium-calmodulin and is potentiated by tropomyosin. Interacts with actin, myosin, two molecules of tropomyosin and with calmodulin. Also play an essential role during cellular mitosis and receptor capping.
Tissue specificity	High-molecular-weight caldesmon (isoform 1) is predominantly expressed in smooth muscles, whereas low-molecular-weight caldesmon (isoforms 2, 3, 4 and 5) are widely distributed in non-muscle tissues and cells. Not expressed in skeletal muscle or heart.
Sequence similarities	Belongs to the caldesmon family.
Domain	The N-terminal part seems to be a myosin/calmodulin-binding domain, and the C-terminal a tropomyosin/actin/calmodulin-binding domain. These two domains are separated by a central helical region in the smooth-muscle form.
Post-translational modifications	In non-muscle cells, phosphorylation by CDK1 during mitosis causes caldesmon to dissociate from microfilaments. Phosphorylation reduces caldesmon binding to actin, myosin, and calmodulin as well as its inhibition of actomyosin ATPase activity. Phosphorylation also occurs in

	both quiescent and dividing smooth muscle cells with similar effects on the interaction with actin and calmodulin and on microfilaments reorganization.
Cellular localization	Cytoplasm > cytoskeleton. Cytoplasm > myofibril. On thin filaments in smooth muscle and on stress fibers in fibroblasts (nonmuscle).

Images



Western blot - Anti-Caldesmon/CDM antibody [E89] (ab32330)

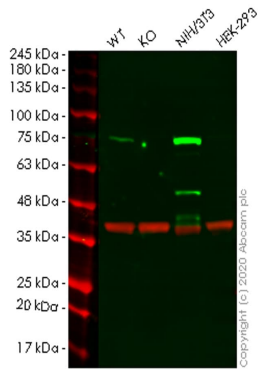
All lanes : Anti-Caldesmon/CDM antibody [E89] (ab32330) at 1/10000 dilution (Purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate
Lane 3 : Rat liver lysate

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: 93 kDa



Western blot - Anti-Caldesmon/CDM antibody [E89] (ab32330)

All lanes : Anti-Caldesmon/CDM antibody [E89] (ab32330) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : CALD1 knockout HeLa cell lysate
Lane 3 : NIH/3T3 cell lysate
Lane 4 : HEK-293 cell lysate

Lysates/proteins at 20 µg per lane.

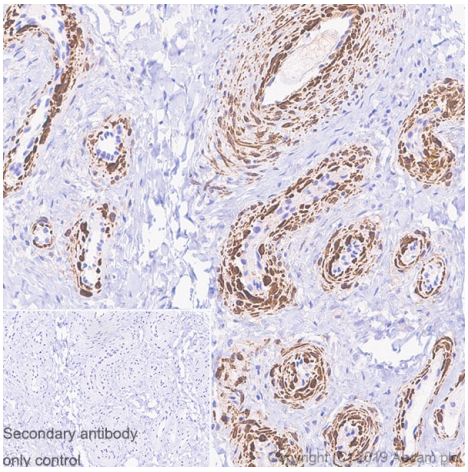
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 93 kDa
Observed band size: 75 kDa

Lanes 1-4: Merged signal (red and green). Green - ab32330

observed at 75 kDa. Red - loading control **ab8245** observed at 36 kDa.

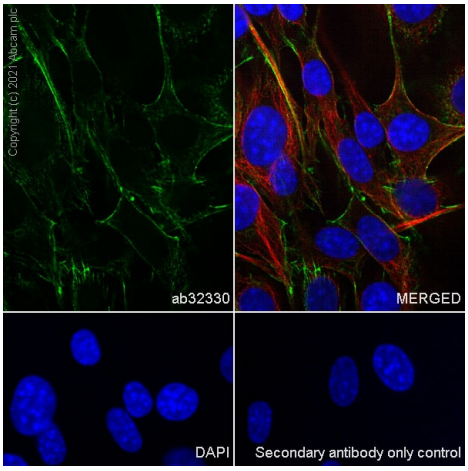
ab32330 Anti-Caldesmon/CDM antibody [E89] was shown to specifically react with Caldesmon/CDM in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265026** (knockout cell lysate **ab257375**) was used. Wild-type and Caldesmon/CDM knockout samples were subjected to SDS-PAGE. ab32330 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) 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® 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caldesmon/CDM antibody [E89] (ab32330)

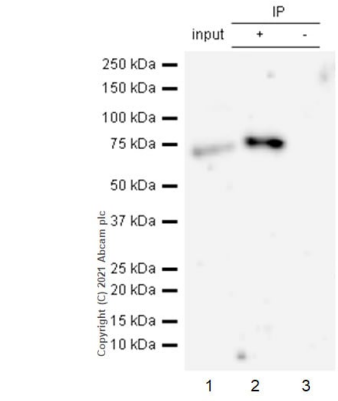
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human leiomyoma tissue sections labeling Caldesmon/CDM with purified ab32330 at 1/100 dilution (1.21 µg/mL). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (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.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument



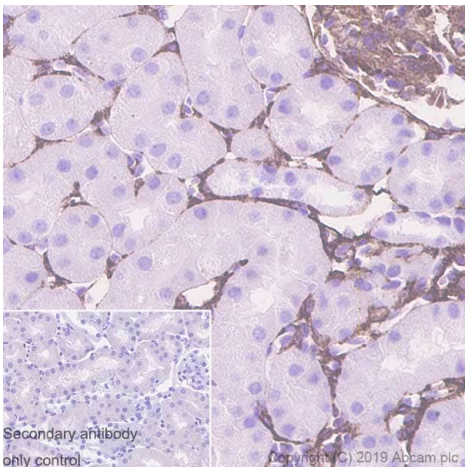
Immunocytochemistry/ Immunofluorescence - Anti-Caldesmon/CDM antibody [E89] (ab32330)

Immunocytochemistry analysis of NIH/3T3 (Mouse embryonic fibroblast) cells labeling Caldesmon/CDM with purified ab32330 at 1/50 dilution (2.4 µg/mL). Cells were fixed in 100% Methanol 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.



Immunoprecipitation - Anti-Caldesmon/CDM antibody [E89] (ab32330)

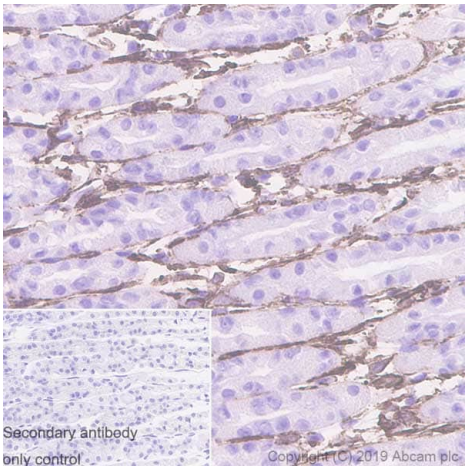
Purified ab32330 at 1/20 dilution (0.6µg) immunoprecipitating Caldesmon/CDM in NIH/3T3 whole cell lysate.
Lane 1 (input): NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate.
Lane 2 (+): ab32330 + NIH/3T3 whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32330 in NIH/3T3 whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (**ab131366**) (1/1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caldesmon/CDM antibody [E89] (ab32330)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue sections labeling Caldesmon/CDM with purified ab32330 at 1/100 dilution (1.21 µg/mL). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (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.

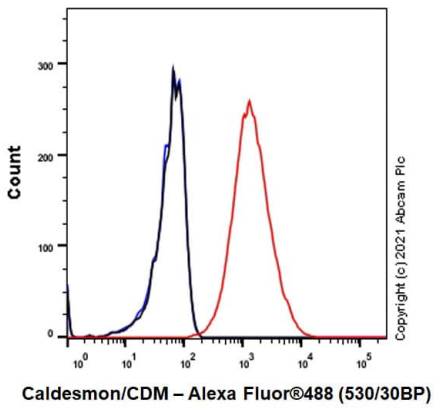
The immunostaining was performed on a Leica Biosystems BOND® RX instrument



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caldesmon/CDM antibody [E89] (ab32330)

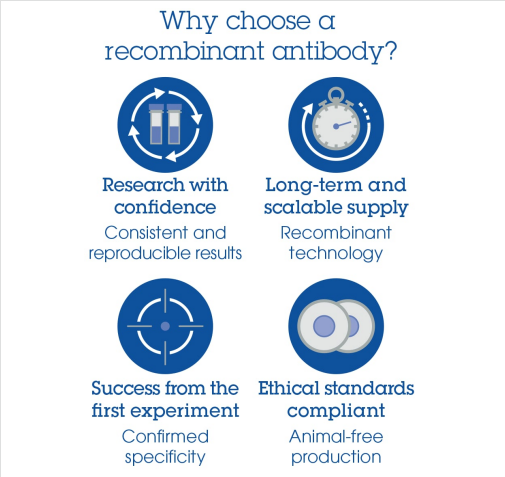
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat stomach tissue sections labeling Caldesmon/CDM with purified ab32330 at 1/100 dilution (1.21 µg/mL). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (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.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument



Flow Cytometry (Intracellular) - Anti-Caldesmon/CDM antibody [E89] (ab32330)

Intracellular Flow Cytometry analysis of NIH/3T3 (Mouse embryonic fibroblast) cells labelling Caldesmon/CDM with purified ab32330 at 1/20 dilution (10 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Anti-Caldesmon/CDM antibody [E89] (ab32330)

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