abcam

Product datasheet

Anti-Caldesmon/CDM antibody [E89] ab32330



יעלטעבע RabMAb

27 References 画像数9

製品の概要

製品名 Anti-Caldesmon/CDM antibody [E89]

製品の詳細 Rabbit monoclonal [E89] to Caldesmon/CDM

由来種 Rabbit

アプリケーション 適用あり: WB, IHC-P, Flow Cyt (Intra), IP, ICC/IF

種交差性 交差種: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール 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.

特記事項 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**.

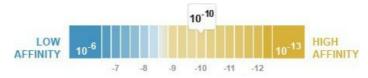
製品の特性

製品の状態 Liquid

保存方法 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.

 $K_D = 1.25 \times 10^{-10} M$ 解離定数(K_D値)



Learn more about K_D

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

精製度 Protein A purified

ポリ/モノ モノクローナル

ウローン名 E89 **アイソタイプ** IgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab32330の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
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 lgG, 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.

ターゲット情報

機能 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.

組織特異性 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.

配列類似性 Belongs to the caldesmon family.

ドメイン 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.

翻訳後修飾 In non-muscle cells, phosphorylation by CDK1 during mitosis causes caldesmon to dissociate

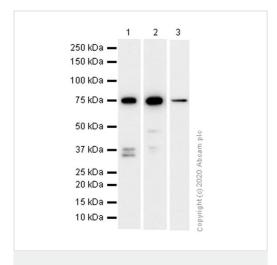
from microfilaments. Phosphorylation reduces caldesmon binding to actin, myosin, and

calmodulin as well as its inhibition of actomyosin ATPase activity. Phosphorylation also occurs in

細胞内局在

Cytoplasm > cytoskeleton. Cytoplasm > myofibril. On thin filaments in smooth muscle and on stress fibers in fibroblasts (nonmuscle).

画像



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

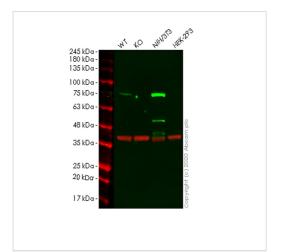
Lane 3: Rat liver lysate

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) 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

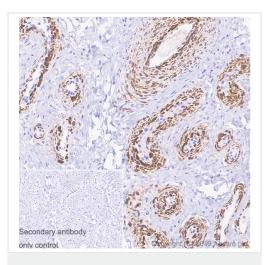
All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) at 1/10000 dilution

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.

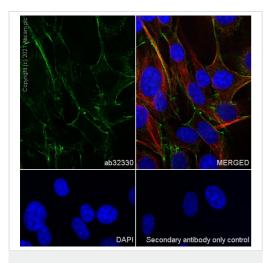
ab32330 Anti-Caldesmon/CDM antibody [E89] was shown to specifically react with Caldesmon/CDM in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265026 (knockout cell lysate ab257375) was used. Wild-type and Caldesmon/CDM knockout samples were subjected to SDS-PAGE. ab32330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

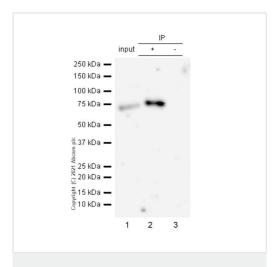
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human leiomyoma tissue sections labeling Caldesmon/CDM with purified ab32330 at 1/100 dilution (1.21 µg/mL). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval 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 μ g/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 μ g/mL). Goat anti rabbit lgG (Alexa Fluor® 488, <u>ab150077</u>) was used as the secondary antibody at 1/1000 (2 μ g/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.

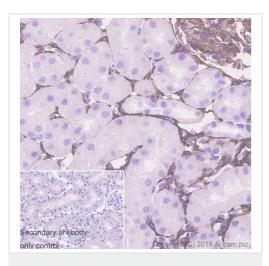
Lane 2 (+): ab32330 + NIH/3T3 whole cell lysate.

Lane 3 (-): Rabbit monoclonal $\lg G$ (ab172730) instead of ab32330 in NIH/3T3 whole cell lysate.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) (1/1000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

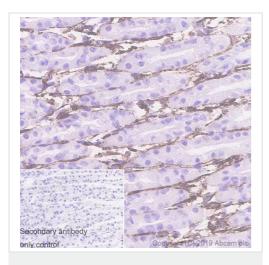
Diluting buffer and concentration: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

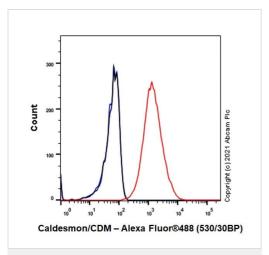
The immunostaining was performed on a Leica Biosystems BOND® RX instrument



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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 μ g/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit lgG (Alexa Fluor[®] 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



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