abcam

Product datasheet

Anti-Annexin-6/ANXA6 antibody [EPR19536] ab201024

KO VALIDATED Recombinant RabMAb

★★★★★ <u>1 Abreviews</u> 10 Images

Overview

Anti-Annexin-6/ANXA6 antibody [EPR19536]
Rabbit monoclonal [EPR19536] to Annexin-6/ANXA6
Rabbit
Suitable for: WB, ICC/IF, IP, IHC-P, Flow Cyt (Intra)
Reacts with: Human
This product was produced with the following immunogens:
Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
WB: Human fetal liver, fetal heart, fetal kidney, spleen and skeletal muscle lysates; Jurkat, Raji, HeLa and HepG2 whole cell lysates. IHC-P: Human tonsil and pancreas tissues. ICC/IF: HepG2 and Raji cells. Flow Cyt (intra): HepG2 cells. IP: Human fetal liver whole cell lysate.
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.

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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.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR19536
lsotype	lgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab201024 in the following tested applications.

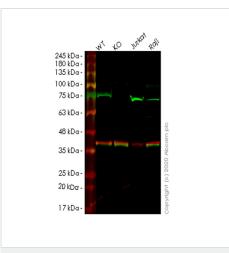
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★ ★ ★ ★ ★ (1)	1/1000. Detects a band of approximately 72 kDa (predicted molecular weight: 76 kDa).
ICC/IF		1/500.
IP		1/30.
IHC-P		1/8000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/600.

Target

Function	May associate with CD21. May regulate the release of Ca(2+) from intracellular stores.
Sequence similarities	Belongs to the annexin family. Contains 8 annexin repeats.
Domain	A pair of annexin repeats may form one binding site for calcium and phospholipid.
Post-translational modifications	Phosphorylated in response to growth factor stimulation.
Cellular localization	Cytoplasm. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

Images



Western blot - Anti-Annexin-6/ANXA6 antibody [EPR19536] (ab201024) **All lanes :** Anti-Annexin-6/ANXA6 antibody [EPR19536] (ab201024) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate Lane 2 : ANXA6 knockout HeLa cell lysate Lane 3 : Jurkat cell lysate Lane 4 : Raji cell lysate

Lysates/proteins at 20 µg per lane.

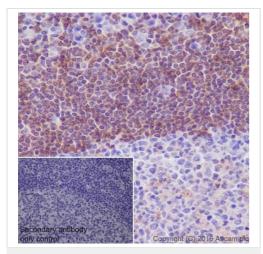
Secondary

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

Predicted band size: 76 kDa Observed band size: 75 kDa

Lanes 1-4: Merged signal (red and green). Green - ab201024 observed at 75 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

ab201024 Anti-Annexin-6/ANXA6 antibody [EPR19536] was shown to specifically react with Annexin-6/ANXA6 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265677** (knockout cell lysate **ab257351**) was used. Wild-type and Annexin-6/ANXA6 knockout samples were subjected to SDS-PAGE. ab201024 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at room temperature for 2. 5 hours 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.



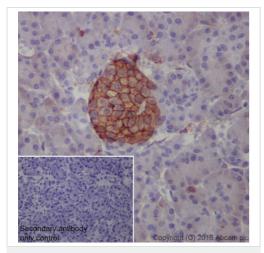
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Annexin-6/ANXA6 antibody [EPR19536] (ab201024)

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling Annexin-6/ANXA6 with ab201024 at 1/8000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

Cytoplasm staining on Human tonsil is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <u>ab97051</u> at 1/500 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Annexin-6/ANXA6 antibody [EPR19536] (ab201024)

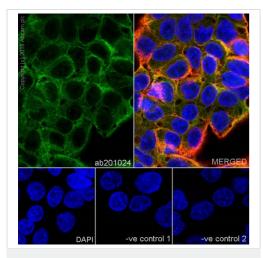
Immunohistochemical analysis of paraffin-embedded Human pancreas tissue labeling Annexin-6/ANXA6 with ab201024 at 1/8000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

Cytoplasm and membrane staining on the Human pancreas islet is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Annexin-6/ANXA6 antibody [EPR19536] (ab201024)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HepG2 (Human liver hepatocellular carcinoma cell line) cells labeling Annexin-6/ANXA6 with ab201024 at 1/500 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor[®] 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on HepG2 cells. The nuclear counterstain is DAPI (blue).

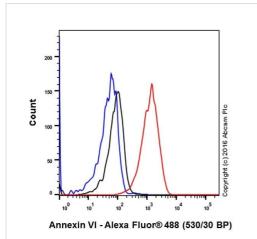
Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] -

Loading Control (<u>**ab7291**</u>) at 1/1000 dilution and Goat Anti-Mouse lgG H&L (Alexa Fluor® 594) preadsorbed (<u>**ab150120**</u>) at 1/1000 dilution (red).

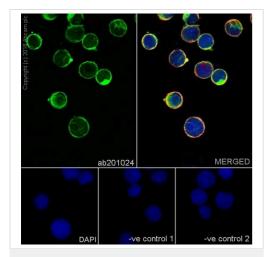
The negative controls are as follows:-

-ve control 1: ab201024 at 1/500 dilution followed by **ab150120** at 1/1000 dilution.

-ve control 2: <u>**ab7291**</u> at 1/1000 dilution followed by <u>**ab150077**</u> at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-Annexin-6/ANXA6 antibody [EPR19536] (ab201024) Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HepG2 (Human liver hepatocellular carcinoma cell line) cells labeling Annexin-6/ANXA6 with ab201024 at 1/600 dilution (red) compared with a a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit IgG (Alexa Fluorr[®] 488) at 1/2000 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Annexin-6/ANXA6 antibody [EPR19536] (ab201024) Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Raji (Human Burkitt's lymphoma cell line) cells labeling Annexin-6/ANXA6 with ab201024 at 1/500 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor[®] 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on Raji cells.

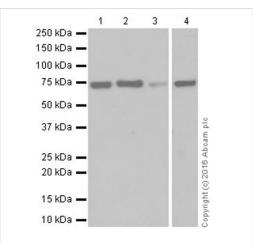
The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] -Loading Control (**ab7291**) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab201024 at 1/500 dilution followed by **ab150120** at 1/1000 dilution.

-ve control 2: <u>**ab7291**</u> at 1/1000 dilution followed by <u>**ab150077**</u> at 1/1000 dilution.



Western blot - Anti-Annexin-6/ANXA6 antibody [EPR19536] (ab201024) **All lanes :** Anti-Annexin-6/ANXA6 antibody [EPR19536] (ab201024) at 1/1000 dilution

Lane 1 : Human fetal liver lysate Lane 2 : Human fetal heart lysate Lane 3 : Human fetal kidney lysate Lane 4 : Human spleen lysate

Lysates/proteins at 10 µg per lane.

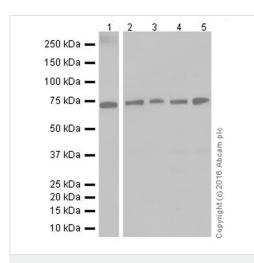
Secondary

All lanes : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 76 kDa Observed band size: 72 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1,2 and 3:5 seconds; Lane 4: 30 seconds.



Western blot - Anti-Annexin-6/ANXA6 antibody [EPR19536] (ab201024) **All lanes :** Anti-Annexin-6/ANXA6 antibody [EPR19536] (ab201024) at 1/5000 dilution

Lane 1 : Human skeletal muscle lysate
Lane 2 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate
Lane 3 : Raji (Human Burkitt's lymphoma cell line) whole cell lysate
Lane 4 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 5 : HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

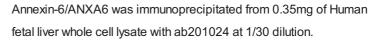
Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 76 kDa Observed band size: 72 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 3 minutes; Lane 2,3,4 and 5: 30 seconds.



Western blot was performed from the immunoprecipitate using ab201024 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

Lane 1: Human fetal liver whole cell lysate,10µg (Input).

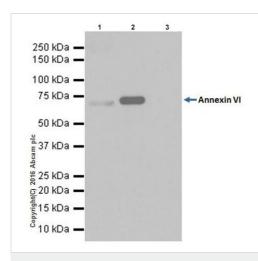
Lane 2: ab201024 IP in Human fetal liver whole cell lysate.

Lane 3: Rabbit IgG, monoclonal [EPR25A] - Isotype Control

(ab172730) instead of ab201024 in Human fetal liver whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 seconds.



Immunoprecipitation - Anti-Annexin-6/ANXA6 antibody [EPR19536] (ab201024)



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