

# **Product datasheet**

# Anti-Annexin-6/ANXA6 antibody [EPR17308] - Nterminal ab199422

KO VALIDATED Recombinant RobMAb

7 Images

Properties

Overview	
Product name	Anti-Annexin-6/ANXA6 antibody [EPR17308] - N-terminal
Description	Rabbit monoclonal [EPR17308] to Annexin-6/ANXA6 - N-terminal
Host species	Rabbit
Tested applications	Suitable for: IP, ICC/IF, WB
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Raji, HeLa and Jurkat whole cell lysate. Mouse brain, kidney and spleen tissue lysate. Rat brain, heart, kidney and spleen tissue lysate. ICC/IF: HeLa and K562 cells.
General notes	<ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <li>High batch-to-batch consistency and reproducibility</li> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> <li>Animal-free production</li> <li>For more information <u>see here</u>.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u>.</li> </ul>

Properues	
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	EPR17308

# Applications

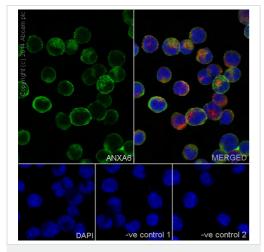
 The Abpromise guarantee
 Our Abpromise guarantee
 covers the use of ab199422 in the following tested applications.

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

Application	Abreviews	Notes
IP		Use at an assay dependent concentration.
ICC/IF		1/100.
WB		1/1000. Detects a band of approximately 68 kDa (predicted molecular weight: 76 kDa).

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

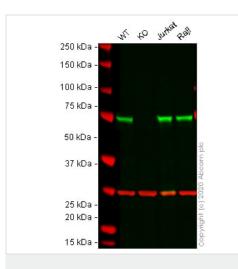


Immunocytochemistry/ Immunofluorescence - Anti-Annexin-6/ANXA6 antibody [EPR17308] - N-terminal (ab199422) Immunofluorescent analysis of 100% methanol-fixed, 0.1% Triton X-100 permeabilized K562 (Human chronic myelogenous leukemia cells from bone marrow) cells labeling Annexin-6/ANXA6 with ab199422 at 1/100, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 (green). Cytoplasm staining on K562 cell line is observed (Subcellular Location: Cytoplasm [UniProt]). The nuclear counterstain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 (red).

The negative controls are as follows:-

-ve control 1 - ab199422 at 1/1000 followed by <u>ab150120</u>
(AlexaFluor®594 Goat anti-Mouse secondary) at 1/500.
-ve control 2. - <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000
followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit IgG

#### H&L) at 1/500.



Western blot - Anti-Annexin-6/ANXA6 antibody [EPR17308] - N-terminal (ab199422)

All lanes : Anti-Annexin-6/ANXA6 antibody [EPR17308] - Nterminal (ab199422) 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

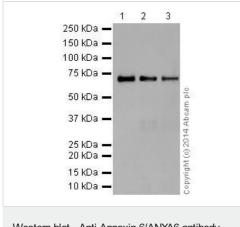
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

Lanes 1 - 4: Merged signal (red and green). Green - ab199422 observed at 75 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab199422 was shown to react with Annexin-6/ANXA6 in HeLa wildtype cells in western blot with loss of signal observed in ANXA6 knockout cell line <u>ab265677</u> (ANXA6 knockout cell lysate <u>ab257351</u>). Wild-type and ANXA6 knockout HeLa cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween<sup>®</sup>) before incubation with ab199422 and <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Annexin-6/ANXA6 antibody [EPR17308] - N-terminal (ab199422) All lanes : Anti-Annexin-6/ANXA6 antibody [EPR17308] - Nterminal (ab199422) at 1/10000 dilution

Lane 1 : Raji (Human Burkitt's lymphoma) whole cell lysate Lane 2 : HeLa (Human epithelial cells from cervix adenocarcinoma ) whole cell lysate

Lane 3 : Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate

Lysates/proteins at 20 µg per lane.

# Secondary

**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

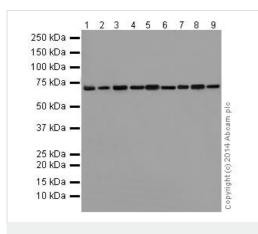
Developed using the ECL technique.

Predicted band size: 76 kDa Observed band size: 68 kDa

Exposure time: 15 seconds

Blocking and diluting buffer was 5% NFDM/TBST.

The observed MW is consistent with what has been described in the literature (PMID:12140262).



Western blot - Anti-Annexin-6/ANXA6 antibody [EPR17308] - N-terminal (ab199422) All lanes : Anti-Annexin-6/ANXA6 antibody [EPR17308] - Nterminal (ab199422) at 1/1000 dilution

- Lane 1 : Mouse brain tissue lysate
- Lane 2 : Mouse kidney tissue lysate
- Lane 3 : Mouse spleen tissue lysate
- Lane 4 : Rat brain tissue lysate
- Lane 5 : Rat heart tissue lysate
- Lane 6 : Rat kidney tissue lysate
- Lane 7 : Rat spleen tissue lysate
- Lane 8 : PC-12 (Rat adrenal gland pheochromocytoma) cell lysate
- Lane 9 : NIH/3T3 (Mouse embryo fibroblast) whole cell lysate

Lysates/proteins at 10 µg per lane.

# Secondary

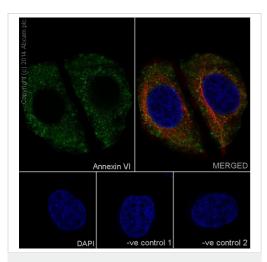
**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Developed using the ECL technique.

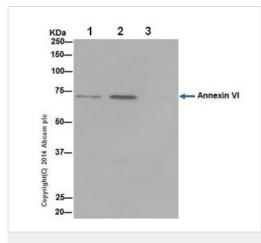
Predicted band size: 76 kDa Observed band size: 68 kDa

Exposure time: 15 seconds

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Immunocytochemistry/ Immunofluorescence - Anti-Annexin-6/ANXA6 antibody [EPR17308] - N-terminal (ab199422)



Immunoprecipitation - Anti-Annexin-6/ANXA6 antibody [EPR17308] - N-terminal (ab199422) Immunofluorescent analysis of 100% methanol-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Annexin-6/ANXA6 with ab199422 at 1/100, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 (green). Cytoplasm staining on HeLa cell line is observed (Subcellular Location: Cytoplasm [UniProt]). The nuclear counterstain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 (red).

The negative controls are as follows:-

-ve control 1 - ab199422 at 1/1000 followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500.
-ve control 2. - <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/500.

Annexin-6/ANXA6 was immunoprecipitated from 1mg of Raji (Human Burkitt's lymphoma cell line) whole cell extract with ab199422 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab199422 at 1/1000 dilution. Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: Raji whole cell extract 10 µg (Input).

Lane 2: ab199422 IP in Raji whole cell extract.

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab199422 in Raji whole cell extract.

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



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