abcam

Product datasheet

Anti-N WASP antibody [EPR6959] ab126626





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Overview

Product name Anti-N WASP antibody [EPR6959]

Description Rabbit monoclonal [EPR6959] to N WASP

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, IHC-P, ICC/IF

Species reactivity Reacts with: Mouse, Human

Predicted to work with: Pig

Immunogen Synthetic peptide within Human N WASP aa 300-450. The exact sequence is proprietary.

Positive control NIH 3T3, F9, L929, HeLa and K562 cell lysates; Human papillary carcinoma tissue.

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**.

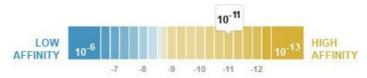
Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Stable for 12 months at -20°C.

Dissociation constant (K_D) $K_D = 7.40 \times 10^{-11} M$



Learn more about K_D

Storage buffer pH: 7.20 Preservative: 0.01% Sodium azide

Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EPR6959

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab126626 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
Flow Cyt (Intra)		Use at an assay dependent concentration. Purified format
WB	★★★★ (1)	1/1000 - 1/10000. Detects a band of approximately 65 kDa (predicted molecular weight: 55 kDa).
IHC-P	****(1)	1/50 - 1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		1/300. For unpurified, use 1/50 - 1/100.

Target

Function Regulates actin polymerization by stimulating the actin-nucleating activity of the Arp2/3 complex.

Binds to HSF1/HSTF1 and forms a complex on heat shock promoter elements (HSE) that

negatively regulates HSP90 expression.

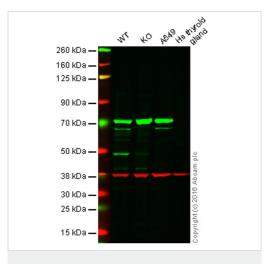
Sequence similarities Contains 1 CRIB domain.

Contains 1 WH1 domain. Contains 2 WH2 domains.

Cytoplasm > cytoskeleton. Nucleus. Preferentially localized in the cytoplasm when phosphorylated

and in the nucleus when unphosphorylated.

Images



Western blot - Anti-N WASP antibody [EPR6959] (ab126626)

Lane 1: Wild-type HAP1 cell lysate (20 µg)

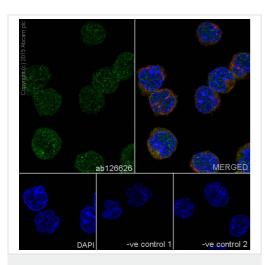
Lane 2: N WASP knockout HAP1 cell lysate (20 µg)

Lane 3: A549 cell lysate (20 µg)

Lane 4: Human thyroid tissue lysate (20 µg)

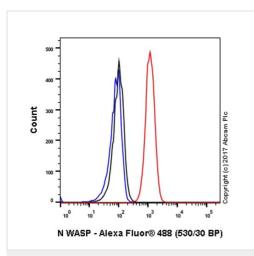
Lanes 1 - 4: Merged signal (red and green). Green - ab126626 observed at 67 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab126626 was shown to recognize N WASP when N WASP knockout samples were used, along with additional cross-reactive bands. Wild-type and N WASP knockout samples were subjected to SDS-PAGE. ab126626 and ab8245 (loading control to GAPDH) were diluted 1/1000 and 1/2000 respectively and incubated overnight at 4°C. 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 1/10000 dilution for 1 h at room temperature before imaging.



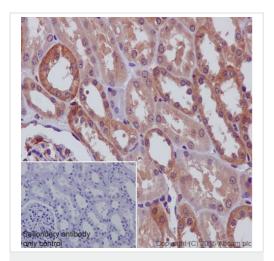
Immunocytochemistry/ Immunofluorescence - Anti-N WASP antibody [EPR6959] (ab126626)

Immunofluorescence staining of K562 cells with purified ab126626 at a working dilution of 1/300, counter-stained with DAPI. The secondary antibody was Alexa Fluor[®] 488 goat anti-rabbit (ab150077), used at a dilution of 1/1000. ab7291, a mouse antitubulin antibody (1/1000), was used to stain tubulin along with ab150120 (Alexa Fluor[®] 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab126626 was used at a dilution of 1/500 followed by an Alexa Fluor[®] 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500. For negative control 2, ab7291 (mouse antitubulin) was used at a dilution of 1/500 followed by an Alexa Fluor[®] 488 goat anti-rabbit antibody (ab150077) at a dilution of 1/400.



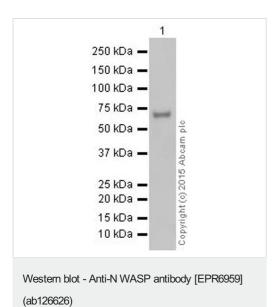
Flow Cytometry (Intracellular) - Anti-N WASP antibody [EPR6959] (ab126626)

Intracellular Flow Cytometry analysis of K562 (human chronic myelogenous leukemia) cells labeling N WASP with purified ab126626 at 1/150 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat Anti-Rabbit IgG H&L (Alexa Fluorr® 488) (ab150077) was used as the secondary antibody. Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-N WASP antibody
[EPR6959] (ab126626)

Immunohistochemical staining of paraffin embedded human kidney with purified ab126626 at a working dilution of 1/100. The secondary antibody used is **ab97051**, a goat anti-rabbit IgG (H&L) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

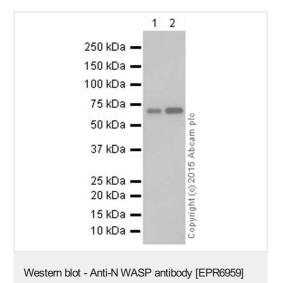


Anti-N WASP antibody [EPR6959] (ab126626) at 1/10000 dilution (purified) + L929 whole cell lysate at 10 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 55 kDa **Observed band size:** 65 kDa



(ab126626)

All lanes : Anti-N WASP antibody [EPR6959] (ab126626) at 1/10000 dilution (purified)

Lane 1 : HeLa whole cell lysate
Lane 2 : K562 whole cell lysate

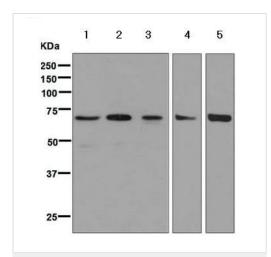
Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 55 kDa **Observed band size:** 65 kDa

Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST



Western blot - Anti-N WASP antibody [EPR6959] (ab126626)

All lanes : Anti-N WASP antibody [EPR6959] (ab126626) at 1/1000 dilution (Unpurified)

Lane 1: NIH 3T3 cell lysate

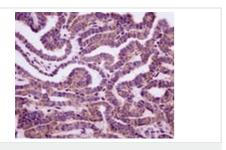
Lane 2: F9 cell lysate
Lane 3: L929 cell lysate
Lane 4: HeLa cell lysate
Lane 5: K562 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat anti-rabbit HRP conjugated antibody at 1/2000 dilution

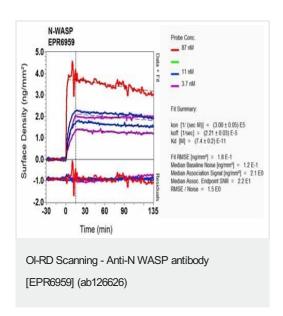
Predicted band size: 55 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-N WASP antibody
[EPR6959] (ab126626)

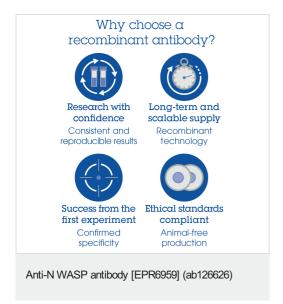
Unpurified ab126626, at 1/50, staining N WASP in formalin fixed, paraffin embedded human papillary carcinoma tissue by Immunohistochemistry

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Equilibrium disassociation constant (K_D) Learn more about K_D

Click here to learn more about K_D



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