abcam

ab308066

Anti-RNF5/NG2 antibody [EPR27133-30]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR27133-30
Purification technique	Affinity purification Protein A
Concentration	0.542 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human
Dilution info	1/1000
Notes	The observed MW of WB-3 is 45 kDa because the sample is recombinant protein.

Not recommended

Species	Mouse
Dilution info	-
Notes	The observed MW of WB-3 is 45 kDa because the sample is recombinant protein.

Species	Rat
Dilution info	-
Notes	The observed MW of WB-3 is 45 kDa because the sample is recombinant protein.

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/50
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

ICC/IF

Not recommended

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

Target data

See full target information RNF5

Function

Membrane-bound E3 ubiquitin-protein ligase that mediates ubiquitination of target proteins (PubMed:11329381, PubMed:12861019, PubMed:16176924, PubMed:19269966, PubMed:19285439). May function together with E2 ubiquitin-conjugating enzymes UBE2D1/UBCH5A and UBE2D2/UBC4 (PubMed:11329381). Mediates ubiquitination of PXN/paxillin,thereby regulating cell motility and localization of PXN/paxillin (PubMed:12861019). Catalyzes ubiquitination of Salmonella type III secreted protein sopA (PubMed:16176924). Mediates the 'Lys-63'-linked polyubiquitination of JKAMP thereby regulating JKAMP function by decreasing its association with components of the proteasome and ERAD; the ubiquitination appears to involve E2 ubiquitin-conjugating enzyme UBE2N (PubMed:19269966). Mediates the 'Lys-48'-linked polyubiquitination of STING1 at 'Lys-150' leading to its proteasomal degradation; the ubiquitination occurs in mitochondria after viral transfection and regulates antiviral responses (PubMed:19285439). Catalyzes ubiquitination and subsequent degradation of ATG4B, thereby inhibiting autophagy (PubMed:23093945).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks

Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

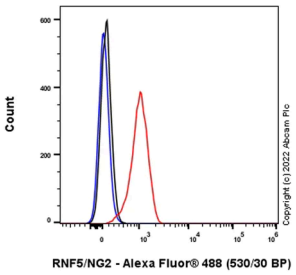
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

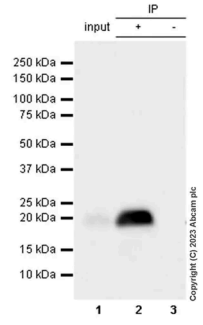
Full details and terms and conditions can be found here:
Terms & Conditions.

6 product images



Flow Cytometry (Intracellular) - Anti-RNF5/NG2 antibody [EPR27133-30] (ab308066)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervical adenocarcinoma epithelial cell) cells labelling RNF5/NG2 with ab308066 at 1/50 dilution (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody. Cells were stained with rabbit IgG or ab308066.



Immunoprecipitation - Anti-RNF5/NG2 antibody [EPR27133-30] (ab308066)

RNF5/NG2 was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10 ug with ab308066 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab308066 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution. Lane 1: HeLa whole cell lysate Lane 2: ab308066 IP in HeLa whole cell lysate Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab308066 in HeLa whole cell lysate Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 10 seconds.
All lanes:
Immunoprecipitation - Anti-RNF5/NG2 antibody [EPR27133-30] (ab308066) at 1/30 dilution

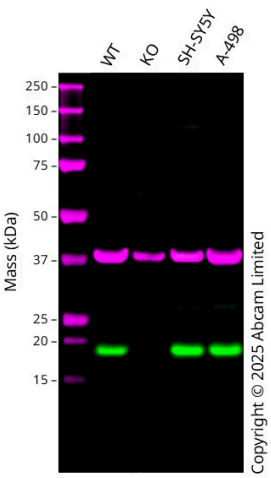
All lanes:
HeLa whole cell lysate at 10 µg

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Observed band size: 19 kDa

Exposure time: 10s



Western blot - Anti-RNF5/NG2 antibody [EPR27133-30] (ab308066)

RNF5/NG2 Western blot staining using rabbit Anti-RNF5/NG2 antibody

Western blot: Rabbit Monoclonal[EPR27133-30] to RNF5/NG2 ab308066 staining at 1/1000 dilution, shown in green; Mouse anti GAPDH (ab8245) loading control staining at 1/20,000 dilution, shown in magenta. A band was observed at 18 kDa in Wild-type A549 cell lysates with no signal observed at this size in RNF5 knockout A549 cell line (ab326022). To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3pc Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-RNF5/NG2 antibody [EPR27133-30] (ab308066) at 1/1000 dilution

Lane 1:
Wild-type A549 at 20 µg

Lane 2:
Western blot - Human RNF5 knockout A549 cell line (ab326022) at 20 µg

Lane 3:
SH-SY5Y at 20 µg

Lane 4:
A-498 at 20 µg

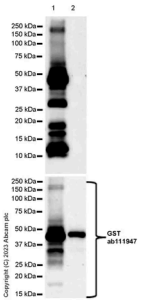
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 20 kDa

Observed band size: 18 kDa



Western blot - Anti-RNF5/NG2 antibody [EPR27133-30] (ab308066)

Blocking and diluting buffer and concentration: 5% NFDM/TBST This antibody does not cross-react with human RNF185. In Western blot, anti-GST antibody (ab111947) staining at 1/5000 dilution.
Exposure time: 180 seconds
All lanes:
Western blot - Anti-RNF5/NG2 antibody [EPR27133-30] (ab308066) at 1/1000 dilution

Lane 1:
Human RNF5 recombinant protein

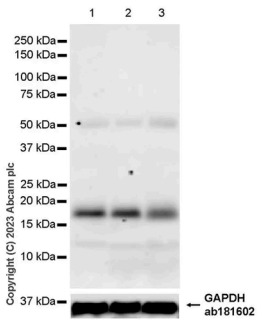
Lane 2:
Human RNF185 recombinant protein

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 45 kDa

Exposure time: 180s



Western blot - Anti-RNF5/NG2 antibody [EPR27133-30] (ab308066)

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
This blot was developed using a high sensitivity ECL substrate. The high-sensitivity ECL substrate used allows for the detection of proteins in the mid-femtogram range.

In Western blot, anti-GAPDH antibody (ab181602) loading control staining at 1/200000 dilution.

All lanes:
Western blot - Anti-RNF5/NG2 antibody [EPR27133-30] (ab308066) at 1/1000 dilution

Lane 1:
Human liver tissue lysate at 20 µg

Lane 2:
Human colon tissue lysate at 20 µg

Lane 3:
Human colon cancer tissue lysate at 20 µg

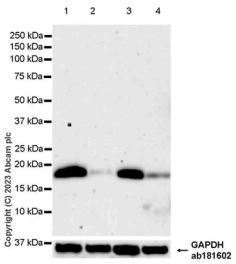
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Observed band size: 19 kDa

Exposure time: 70s



Western blot - Anti-RNF5/NG2 antibody [EPR27133-30] (ab308066)

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
This blot was developed using a high sensitivity ECL substrate. The high-sensitivity ECL substrate used allows for the detection of proteins in the mid-femtogram range.

In Western blot, anti-GAPDH antibody (ab181602) loading control staining at 1/200000 dilution.

All lanes:
Western blot - Anti-RNF5/NG2 antibody [EPR27133-30] (ab308066) at 1/1000 dilution

Lane 1:
293T (human embryonic kidney epithelial cell) transfected with scrambled siRNA control whole cell lysate at 20 µg

Lane 2:
293T transfected with siRNA specifically targeting RNF5/NG2 whole cell lysate at 20 µg

Lane 3:
HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 4:
MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Observed band size: 19 kDa

Exposure time: 180s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.