abcam

Product datasheet

Anti-NG2 antibody [EPR23976-145] ab275024

Recombinant RabMAb

2 References 11 Images

Overview

Product name	Anti-NG2 antibody [EPR23976-145]
Description	Rabbit monoclonal [EPR23976-145] to NG2
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt, ICC/IF, IP, WB **Unsuitable for:** IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human pancreas tissue lysate; Mouse brain and pancreas tissue lysates; rat brain tissue lysate; A375 and SK-MEL-28 whole cell lysates. ICC/IF: Mouse primary neural/glia cells; Rat primary neural/glia cells; LADMAC cells. Flow cyt: LADMAC cells; Mouse primary neural glia cells, A-375 cells. IP: Mouse brain tissue lysate.
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 long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR23976-145

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab275024 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		1/500.
ICC/IF		1/50.
IP		1/30.
WB		1/1000. Detects a band of approximately 280, 330 kDa (predicted molecular weight: 251 kDa).

Application notes Is unsuitable for IHC-P.

Target

Function Proteoglycan playing a role in cell proliferation and migration which stimulates endothelial cells motility during microvascular morphogenesis. May also inhibit neurite outgrowth and growth cone collapse during axon regeneration. Cell surface receptor for collagen alpha 2(VI) which may confer cells ability to migrate on that substrate. Binds through its extracellular N-terminus growth factors, extracellular matrix proteases modulating their activity. May regulate MPP16-dependent degradation and invasion of type I collagen participating in melanoma cells invasion properties. May modulate the plasminogen system by enhancing plasminogen activation and inhibiting angiostatin. Functions also as a signal transducing protein by binding through its cytoplasmic C-terminus scaffolding and signaling proteins. May promote retraction fiber formation and cell polarization through Rho GTPase activation. May stimulate alpha-4, beta-1 integrin-mediated adhesion and spreading by recruiting and activating a signaling cascade through CDC42, ACK1 and BCAR1. May activate FAK and ERK1/ERK2 signaling cascades.

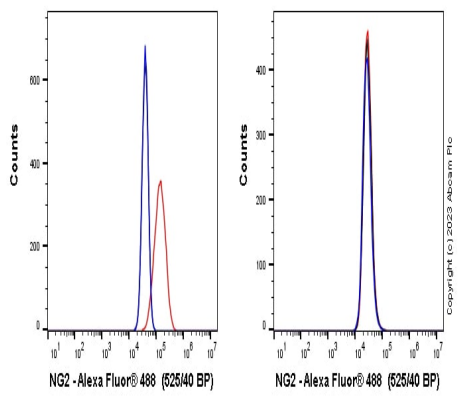
Tissue specificity Detected only in malignant melanoma cells.

Sequence similarities Contains 15 CSPG (NG2) repeats.
Contains 2 laminin G-like domains.

Post-translational modifications O-glycosylated; contains glycosaminoglycan chondroitin sulfate which are required for proper localization and function in stress fiber formation (By similarity). Involved in interaction with MMP16 and ITGA4.
Phosphorylation by PRKCA regulates its subcellular location and function in cell motility.

Cellular localization Apical cell membrane. Cell projection > lamellipodium membrane. Localized at the apical plasma membrane it relocalizes to the lamellipodia of astrocytoma upon phosphorylation by PRKCA. Localizes to the retraction fibers. Localizes to the plasma membrane of oligodendrocytes.

Images



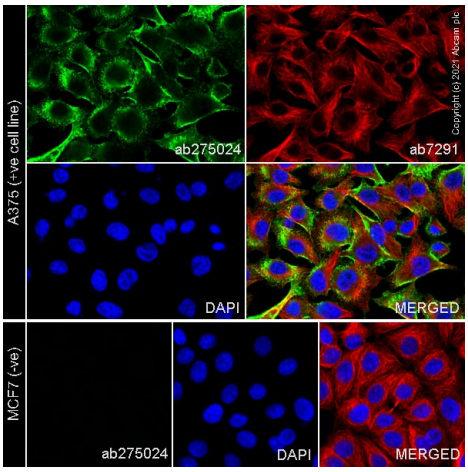
Flow Cytometry - Anti-NG2 antibody [EPR23976-145] (ab275024)

Flow cytometry overlay histogram showing left A-375 positive cells and right negative MCF7 stained with ab275024 (red line). The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interactionfollowed by the antibody (ab275024) (1x 10^6 in 100µl at 5.0 µg/ml (1/402)) for 30min on ice.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min on ice

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

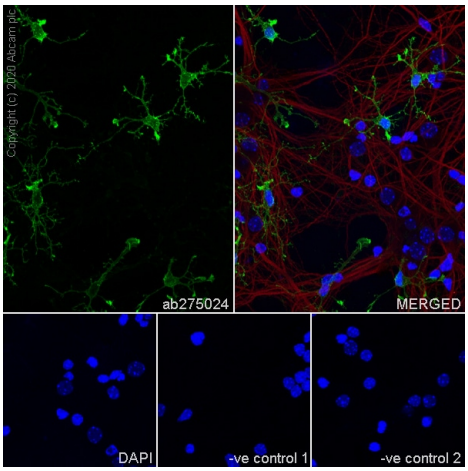


Immunocytochemistry/ Immunofluorescence - Anti-NG2 antibody [EPR23976-145] (ab275024)

ab275024 staining NG2 in A375 cells, with negative expression in MCF7 cells. The cells were fixed with 100% methanol (5 min), permeabilised with 0.1% Triton x-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab275024 at 5 µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin at 0.5 µg/ml. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150119**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 647), pre-adsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.

This product also work with 4% formaldehyde (10 min) fixation under the same testing conditions.

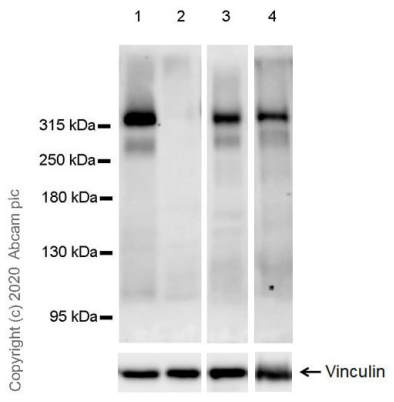


Immunocytochemistry/ Immunofluorescence - Anti-NG2 antibody [EPR23976-145] (ab275024)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized mouse primary neural/glia cell cells labelling NG2 with ab275024 at 1/100 (4.67 ug/ml) dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing positive staining in mouse primary glia cells. Confocal scanning Z step was set as 0.3 µm followed by image processing with maximum Z projection. is observed. **ab11267** Anti-MAP2 mouse monoclonal antibody was used to counterstain MAP2 at 1/500 dilution, followed by **ab150120** Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) at 1/1000 dilution (Red). The Nuclear counterstain was DAPI (Blue).

ve control 1: ab275024 at 1/100 dilution, followed by **ab150120** Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) at 1/1000 dilution.

-ve control 2: **ab11267** at 1/500 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Western blot - Anti-NG2 antibody [EPR23976-145] (ab275024)

All lanes : Anti-NG2 antibody [EPR23976-145] (ab275024) at 1/1000 dilution

Lane 1 : Mouse brain tissue lysate at 20 µg
Lane 2 : Mouse liver tissue lysate at 20 µg
Lane 3 : Mouse pancreas tissue lysate at 40 µg
Lane 4 : Rat brain tissue lysate at 40 µg

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/50000 dilution

Predicted band size: 251 kDa
Observed band size: 280,330 kDa

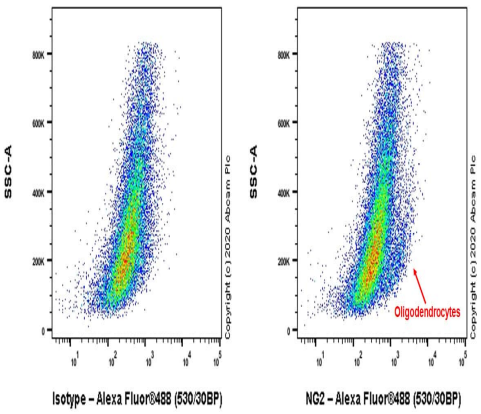
Blocking and dilution buffer: 5% NFDM/TBST.

Exposure times: Lanes 1-3: 59 seconds; Lane 4: 81 seconds.

The band of 330KDa represents the intact NG2 proteoglycan modified by chondroitin sulfate, the band of 280KDa represents NG2 core protein.
The molecular weight observed is consistent with what has been described in the literature (PMID: 20455858, 16625365, 23481707).

Negative control: Mouse liver (PMID: 23481707).

Samples are non-boiled as boiling may cause protein aggregates.

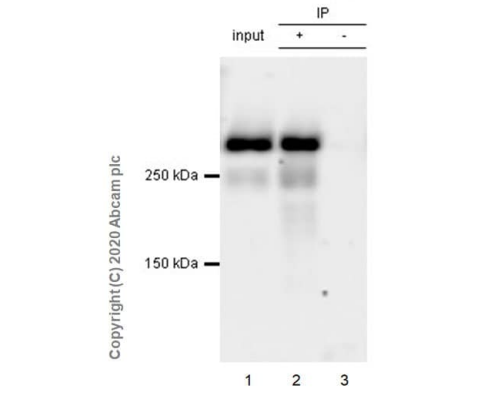


Flow Cytometry - Anti-NG2 antibody [EPR23976-145] (ab275024)

Flow cytometric analysis of Mouse primary neural glia cell cells labelling NG2 with ab275024 at 1/500 dilution (0.1ug) (Right) compared with a Rabbit monoclonal IgG (**ab172730**) isotype control (Left).

Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.

Gated on viable cells.



Immunoprecipitation - Anti-NG2 antibody [EPR23976-145] (ab275024)

NG2 was immunoprecipitated from 0.35 mg Mouse brain tissue lysate with ab275024 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab275024 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP)(**ab131366**) was used at 1/1000 dilution.

Lane 1: Mouse brain tissue lysate 10 ug

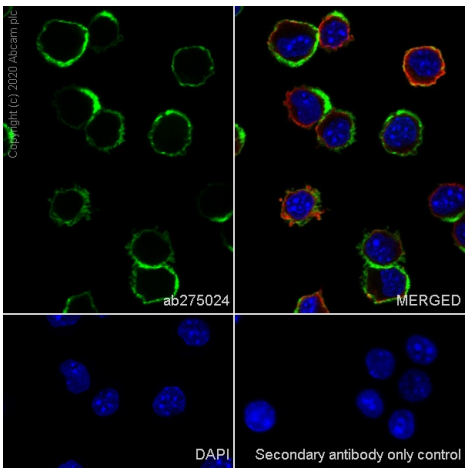
Lane 2: ab275024 IP in Mouse brain tissue lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab275024 in mouse brain tissue lysate

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

Exposure time: 15 seconds.

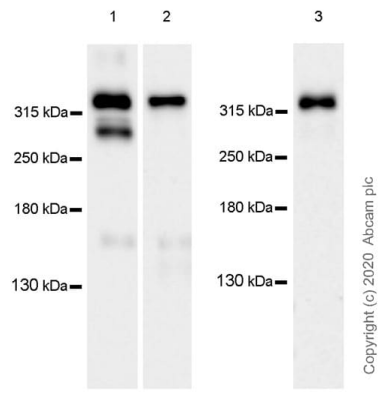
Sample loaded onto lane 1 was non-boiled as boiling may cause protein aggregates.



Immunocytochemistry/ Immunofluorescence - Anti-NG2 antibody [EPR23976-145] (ab275024)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized LADMAC cells labelling NG2 with ab275024 at 1/50 (9.34 ug/ml) dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing membranous staining in LADMAC cell line. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Western blot - Anti-NG2 antibody [EPR23976-145] (ab275024)

All lanes : Anti-NG2 antibody [EPR23976-145] (ab275024) at 1/1000 dilution

Lane 1 : A375 (human malignant melanoma epithelial cell) whole cell lysate at 20 µg
Lane 2 : SK-MEL-28 (human malignant melanoma) whole cell lysate at 20 µg
Lane 3 : Human pancreas tissue lysate at 40 µg

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/50000 dilution

Predicted band size: 251 kDa
Observed band size: 280,330 kDa

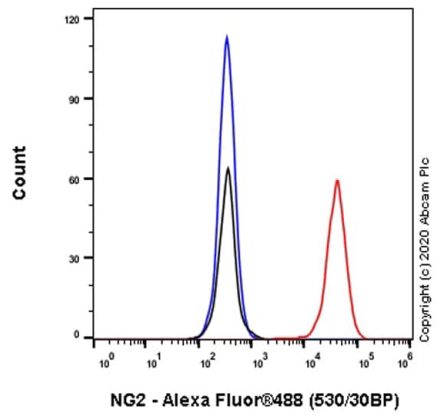
Blocking and dilution buffer: 5% NFDM/TBST.

Exposure times: Lane 1: 26 seconds; Lane 2: 59 seconds; Lane 3: 125 seconds.

The band of 330KDa represents the intact NG2 proteoglycan modified by chondroitin sulfate, the band of 280KDa represents NG2 core protein.

The molecular weight observed is consistent with what has been described in the literature (PMID: 20455858, 16625365, 23481707).

Samples are non-boiled as boiling may cause protein aggregates.

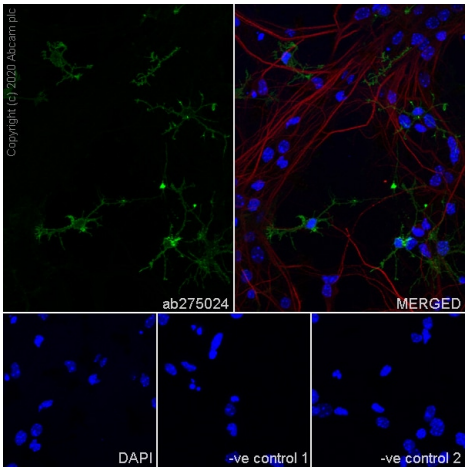


Flow Cytometry - Anti-NG2 antibody [EPR23976-145] (ab275024)

Flow cytometric analysis of LADMAC (Mouse bone marrow monocyte macrophage) cells labelling NG2 with ab275024 at 1/500 dilution (0.1ug) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) isotype control (Black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.

Gated on viable cells.



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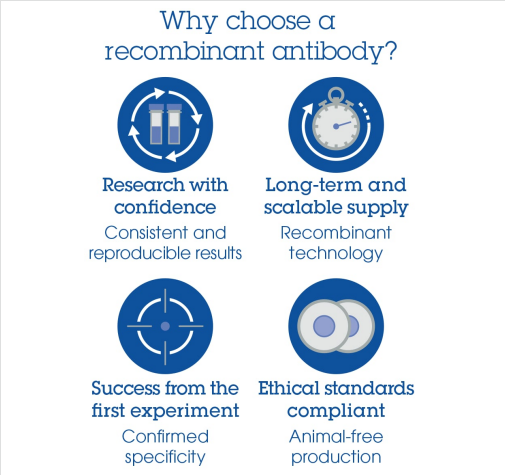
-ve control 1: ab275024 at 1/100 dilution, followed by **ab150120** Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) at 1/1000 dilution.

-ve control 2: **ab11267** at 1/500 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Anti-NG2 antibody [EPR23976-145] (ab275024)

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