abcam

Product datasheet

Anti-NG2 antibody [EPR23976-145] - BSA and Azide free ab275038

Recombinant RabMAb

11 Images

Overview

Product name	Anti-NG2 antibody [EPR23976-145] - BSA and Azide free
Description	Rabbit monoclonal [EPR23976-145] to NG2 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, Flow Cyt, IP, ICC/IF **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	ab275038 is the carrier-free version of **ab275024**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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. Do Not Freeze.
Storage buffer	Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR23976-145
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab275038 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		Use at an assay dependent concentration. Detects a band of approximately 280, 330 kDa (predicted molecular weight: 251 kDa).
Flow Cyt		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.

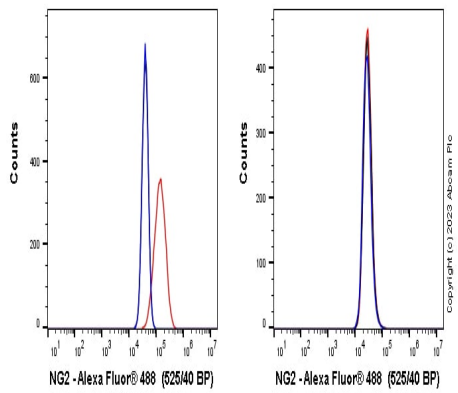
Application notes Is unsuitable for IHC-P.

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] - BSA and Azide free (ab275038)

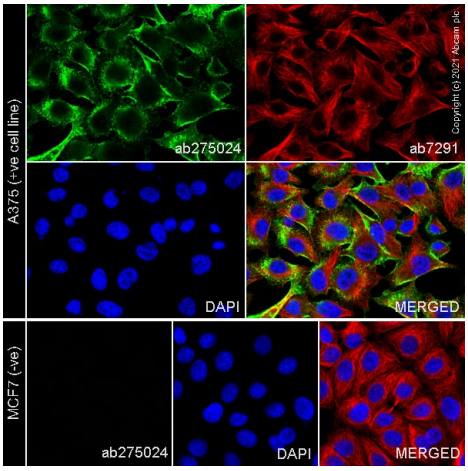
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab275024**).

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.



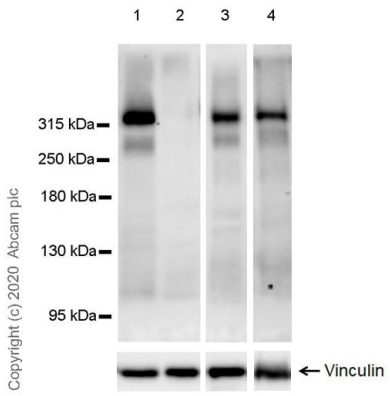
Immunocytochemistry/ Immunofluorescence - Anti-NG2 antibody [EPR23976-145] - BSA and Azide free (ab275038)

This data was developed using **ab275024**, the same antibody clone in a different buffer formulation.

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.



Western blot - Anti-NG2 antibody [EPR23976-145] - BSA and Azide free (ab275038)

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

This data was developed using **ab275024**, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.

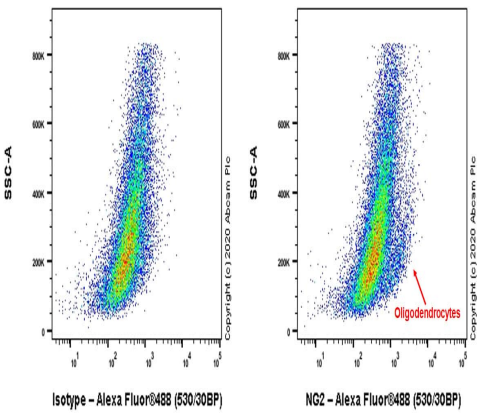
Exposure times: Lanes 1-3: 59 seconds; Lane 4: 81 seconds.

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.



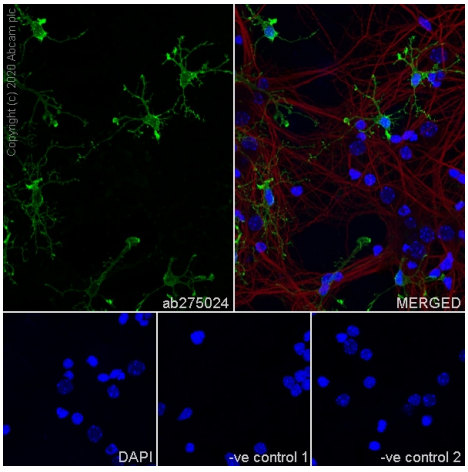
Flow Cytometry - Anti-NG2 antibody [EPR23976-145] - BSA and Azide free (ab275038)

This data was developed using **ab275024**, the same antibody clone in a different buffer formulation.

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.



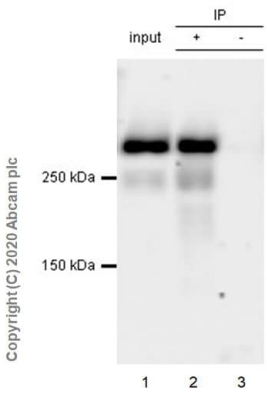
Immunocytochemistry/ Immunofluorescence - Anti-NG2 antibody [EPR23976-145] - BSA and Azide free (ab275038)

This data was developed using **ab275024**, the same antibody clone in a different buffer formulation.

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 counterstainMAP2 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.



Immunoprecipitation - Anti-NG2 antibody [EPR23976-145] - BSA and Azide free (ab275038)

This data was developed using **ab275024**, the same antibody clone in a different buffer formulation.

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

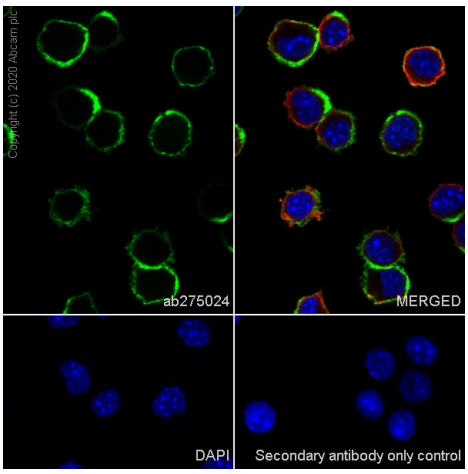
Lane 2: **ab275024** IP in Mouse brain tissue lysate

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.

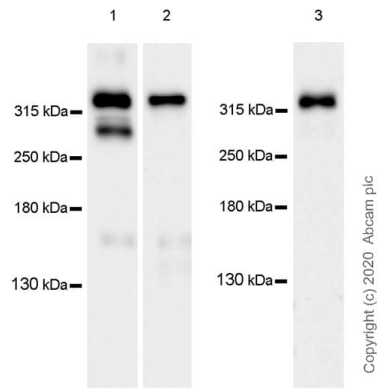


Immunocytochemistry/ Immunofluorescence - Anti-NG2 antibody [EPR23976-145] - BSA and Azide free (ab275038)

This data was developed using **ab275024**, the same antibody clone in a different buffer formulation.

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] - BSA and Azide free (ab275038)

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

This data was developed using **ab275024**, the same antibody clone in a different buffer formulation.

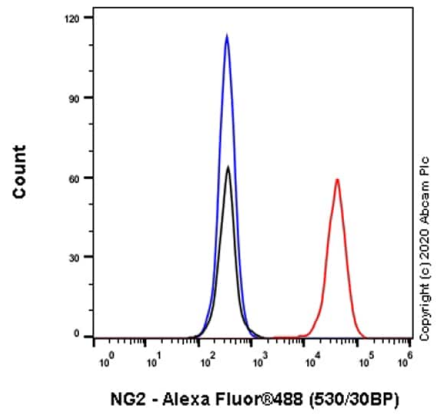
locking and dilution buffer: 5% NFDM/TBST.

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.



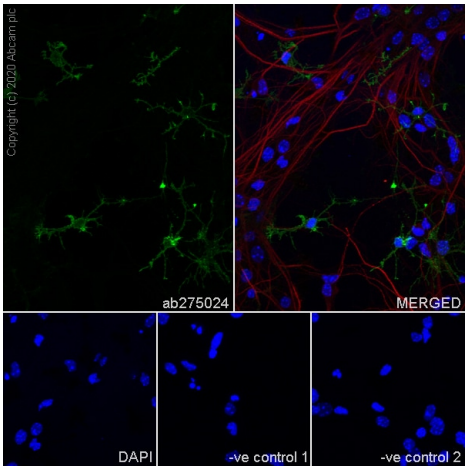
Flow Cytometry - Anti-NG2 antibody [EPR23976-145] - BSA and Azide free (ab275038)

This data was developed using **ab275024**, the same antibody clone in a different buffer formulation.

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.



Immunocytochemistry/ Immunofluorescence - Anti-NG2 antibody [EPR23976-145] - BSA and Azide free (ab275038)

This data was developed using **ab275024**, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized rat 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 rat primary glia cells.Confocal scanning Z step was set as 0.3 µm followed by image processing with maximum Z projection. **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.

Anti-NG2 antibody [EPR23976-145] - BSA and Azide free (ab275038)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors