



MONOCLONAL ANTIBODY

For research use only. Not for clinical diagnosis

Catalog No. PRPG-NG-M01

Anti- NG2 [CSPG4] (2161D7)**BACKGROUND**

NG2, also known as HMW-MAA or MCSP and encoded by the CSPG4 gene, is a unique transmembrane proteoglycan that may be accounted for many of the interactions taking place between cells and their microenvironment during both cell propagation and cell movement. In the adult human body, NG2 has a relatively limited tissue distribution and is characteristically found with prevalence on immature and progenitor cells of various tissues and organs, including brain, skeletal muscle, cartilage and skin. It is generally absent from all mature epithelial and hematopoietic cells, while it becomes de novo expressed upon neoplastic transformation of different cell type with the most striking examples being its appearance on melanocytes converting into melanoma and T lymphocytes and myeloid cells turning into leukemic cells. *

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|-------------------------|--|
| Product type | Primary antibodies |
| Immunogen | Recombinant extracellular domain of human NG2/CSPG4 |
| Rased in | Mouse |
| Myeloma | - |
| Clone number | 2161D7 |
| Isotype | IgG1 |
| Host | - |
| Source | Hybridoma cell culture |
| Purification | - |
| Form | Liquid |
| Storage buffer | Supernatant supplemented with 0.05% NaN ₃ |
| Concentration | ND |
| Volume | 2 mL |
| Label | Unlabeled |
| Specificity | NG2/CSPG4 (epitope not identified) |
| Cross reactivity | Human Other species have not been tested. |
| Storage | Store at 4°C for short-term storage and -20°C for prolonged storage Aliquot to avoid cycles of freeze / thaw. |
| Other | Data Link : UniProtKB/Swiss-Prot CSPG4 HUMAN, Q6UVK1 |

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| Application notes | WB, IP, IHC(P), ELISA |
| Recommended dilutions | Western blotting, 1/10 to 1/30 (Distinct band at 250-260 kD) Immunoprecipitation, 1/5 - 1/10 Immunohistochemistry, 1/5 to 1/50 (paraffin-embedded) * ELISA, 1/10 - 1/150 |

***<Staining Pattern>**

The antibody stains preferentially vascular structures and progenitor cells of skin, cartilage and muscle, oligodendrocyte precursors, certain epithelial cells (precursors); and pericytes and certain tumour cells in melanoma, breast carcinoma and soft-tissue sarcoma lesions.

Other applications have not been tested.

Optimal dilutions/concentrations should be determined by the end user.

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|-------------------|---|
| References | - |
|-------------------|---|

ANTIBODY CHARACTERIZATION

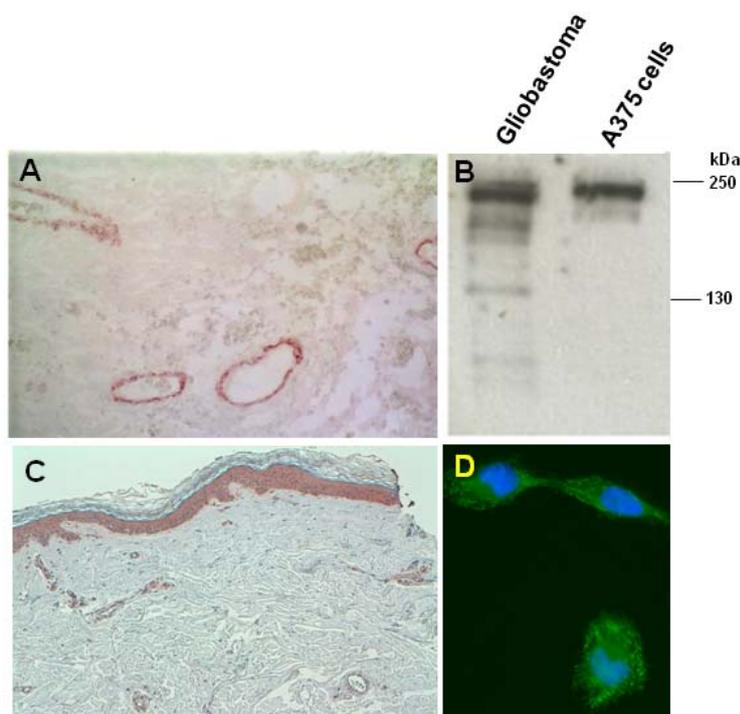


Fig. 1. Immunostainings of a malignant glioblastoma A) and cutaneous melanoma C) lesion. B) Western blotting on whole tissue/cell lysates of human glioblastoma lesion and the human melanoma A375 cell line after SDS-PAGE under reducing conditions on 4-10% gradient gels. D) immunostaining of human melanoma MeWo cells. Nuclear counterstaining was performed with Hoescht.

RELATED PRODUCTS:

| Product Name | Maker | Cat# |
|---|-------|---------------|
| Anti Aggrecan (6F4) Monoclonal Antibody | CAC | PRPG-AG-M01 |
| Anti Aggrecan (5D3) Monoclonal Antibody | CAC | PRPG-AG-M02 |
| Anti Aggrecan (5G2) Monoclonal Antibody | CAC | PRPG-AG-M03 |
| Anti Aggrecan (7B7) Monoclonal Antibody | CAC | PRPG-AG-M04 |
| Anti Versican/CSPG2 (5C12) Monoclonal Antibody | CAC | PRPG-VS-M01 |
| Anti Versican/CSPG2 (4C5) Monoclonal Antibody | CAC | PRPG-VS-M02 |
| Anti NG2 / CSPG4 (2164H5) Monoclonal Antibody | CAC | PRPG-NG-M01 |
| Anti COMP (484D1) Monoclonal Antibody | CAC | PRPG-CP-M01 |
| Anti COMP (490D11) Monoclonal Antibody | CAC | PRPG-CP-M02 |
| Anti Keratan sulfate (373E1) Monoclonal Antibody | CAC | PRPG-KS-M01 |
| Anti Decorin (889C7) Monoclonal Antibody | CAC | PRPG-DC-M01 |
| Anti Fibromodulin (636B12) Monoclonal Antibody | CAC | PRPG-FBM-M01 |
| Anti Biglycan (905A7) Monoclonal Antibody | CAC | PRPG-BG-M01 |
| Anti XTP1 (2191H1) Monoclonal Antibody | CAC | PRPG-XTP-M01 |
| Anti SDP35 (2200D12) Monoclonal Antibody | CAC | PRPG-SDP-M01 |
| Anti Laminin α 4 (652C4) Monoclonal Antibody | CAC | PRPG-LA4-M01 |
| Anti Collagen 12 (378D5) Monoclonal Antibody | CAC | PRPG-CO12-M01 |

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NG2, also known as HMW-MAA or MCSP and encoded by the CSPG4 gene, is a unique transmembrane proteoglycan that may be accounted for many of the interactions taking place between cells and their microenvironment during both cell propagation and cell movement. In the adult human body, NG2 has a relatively limited tissue distribution and is characteristically found with prevalence on immature and progenitor cells of various tissues and organs, including brain, skeletal muscle, cartilage and skin. It is generally absent from all mature epithelial and hematopoietic cells, while it becomes de novo expressed upon neoplastic transformation of different cell type with the most striking examples being its appearance on melanocytes converting into melanoma and T lymphocytes and myeloid cells turning into leukemic cells.

Cloning of the CSPG4 gene, originally accomplished in rat and much later on in man, revealed that the gene is localized on the human chromosome 15:24q2; encodes for a 8.9 kb transcript with an open reading frame of 8,071 nucleotides which translates into a core protein of 2,325 residues encompassing numerous glycosylation sites and three putative glycosaminoglycan (GAG)-attachment sites, of which mostly only one is occupied with a shorter chondroitin sulphate chain with unknown composition. However, NG2 may also behave as a "part-time PG" being in some instances synthesized as a core protein free of GAG chains. In its fully glycosylated form NG2 has an apparent molecular size of >500 kDa, while this isoform often coexists with a number of variants running on SDS-PAGE in the range of 300-400 kDa.

Detailed ultrastructural and sequence analyses of the primary structure of the NG2 ectodomain can be subdivided three distinct subdomains: a globular N-terminal, D3, a flexible rod-like central segment, D2, and a C-terminal one, D1, assuming an extended globular conformation. The D3 subdomain contains 4 phylogenetically conserved Ca²⁺-binding cadherin-type repeats, whereas the membrane proximal one encompasses at least two distinct proteolytic cleavage sites attacked by metalloproteinases that are in part antagonized by TIMP2 and TIMP3. These contribute to both the physiological and injury-associated shedding of the ectodomain from the cell surface in at least two distinct forms having apparent *M_r*s of 290 kDa and 275 kDa, respectively, by a cell-autonomous signal transduction-dependent mechanism. Additional cleavage products of the NG2 ectodomain have been identified in situ in dystrophic human muscle. The cytoplasmic tail of NG2, stretching 76 amino acids, contains two threonine residues prone to differential phosphorylation by PCK□ (Thr2256) and ERK1 (Thr2314), depending on whether the proteoglycan engages in migration or proliferation events. Through the cytoplasmic tail NG2 firmly links to the actin cytoskeleton via bridging of PDZ-type adaptor proteins such MUPP1 and synthenin-1 and close association with ezrin and cofilin-1. Through cytoskeletal connections and the above mentioned PCK□/ERK-dependent threonine phosphorylations, the intracellular domain of NG2 also activates signalling cascades involving FAK, Rac1, cdc42, Ack1 and p130CAS, suggesting that that it may contribute to the execution of intricate patterns of signal transduction governing cytoskeletal dynamics.

The primary extracellular matrix ligands of NG2 known to date are collagens, but recent studies suggest that it may have other binding partners. Additional molecular interactions have been detected with galectin-3, angiostatin and annexin-I during angiogenesis and are thought to be pivotal in the NG2's control of this phenomenon. Both in this situation and on tumour cells, a more direct effect of NG2 on cell adhesion, spreading and motility has been proposed to be exerted through modulation of the function of integrin □3□1 and □4□1 with which NG2 may associate on the cell surface. In glial progenitor cells, NG2 may additionally form complexes with AMPA receptors and PDZ glutamate receptor interaction protein [GRIP].

The presence of NG2 on the cell surface strongly impacts on the growth of the cells and this ability is provided through a well-described docking receptor function of the proteoglycan in cells responding to PDGF-AA and various members of the FGF family. In fact, we find that in NG2 null mice, FGF-elicited corneal angiogenesis is strongly impaired due to a failure of pericytes to undergo normal extension and propagation. Taken together the growth factor coreceptor function of NG2 and its potential of sequestering angiostatin may explain the pivotal support of NG2 pericyte sprouting and tubular formation.

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