ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Nogo-66 receptor (Ngr) Antibodies

Cat. # NGR12-A	Goat Anti-Human Ngr protein IgG # 2, aff pure	**SIZE**: 100 ug
Cat. # NGR12-C	Recombinant Human Ngr-Fc Chimera protein WB +ve control	**SIZE**: 100 ul

Many tissues such as muscle, skin, liver, and peripheral nerve, have remarkable ability to repair and regrow after injury. However, the CNS (brain and spinal cord) is limited in its ability to repair or regrowth causing permanent brain damage or paralyses. Most recently an inhibitory myelin protein, **Nogo (Neurite outgrowth**), has been cloned and characterized. It may help block the regeneration of the CNS. Nogo is the 4th member of **reticulon** (**Rtn**) family. There are three alternative isoforms of Nogo, designated **Nogo-A** (full length), an intermediate form **Nogo-B** (373 aa; ~37 K, lacks 186-1004 aa within the extracellular domain), and a shorter form **Nogo-C** (199 aa; ~25 K, similar to rat vp20 and foocen-s; lacks 186-1004 aa but which has a smaller, alternative N-terminal domain). A 66-aa hydrophilic region of Nogo (**Nogo-66**), located between the two TM domains, has the most inhibitory properties of Nogo. In contrast to Nogo, Rtn 1, -2, and 3 do not inhibit axonal regeneration. This 66-aa region also has the least similarity to Rtn proteins. The corresponding **Rtn-P4** peptide sequence has no activity.

Recently, a brain specific leucine-rich-repeat protein with high affinity for soluble Nogo-66, termed **Nogo receptor** (**Ngr**) has been cloned and characterized. Cleavage of Ngr from the axonal surface renders neurons insensitive to Nogo-66. Ngr expression is sufficient to impart Nogo-66 axonal inhibition to unresponsive neurons. Ngr protein (mouse, rat, monkey, and human 473 aa; chromosome 22q11) contains a signal sequence followed by eight LRR domains, an LRR cysteine-rich CT-flanking domain, and unique GPI anchorage site. Human and mouse Ngr proteins are ~89% identical. Ngr is expressed in brain and lower levels are also detected in hear and kidney but not in other peripheral tissues. Ngr is localized to axons.

Source of Antigen and Antibodies

Human Ngr 27-447 aa (extracellular domain) was fused with human IgG (100-330 aa), expressed as fusion protein in NSO myeloma cells, and purified (95%). Recombinant protein chimera is a disulfide-linked homodimer. Non-glycosylated Ngr-Fc monomer calculated wt is ~72 Kda. Due to glycosylation, Ngr-Fc protein has a mol wt of ~95-100 Kda under reducing conditions.

Source of Antigen and Antibodies

Antigen	Purified human Ngr-Fc protein (extracellular domain)
Ab Host/type	Goat, polyclonal Aff pure IgG (**cat #NGR12-A**) purified over the antigen column
2-ab	Cat # 30220, Rabbit anti-goat IgG-HRP (AP, biotin, FITC conjugates also available
-ve control	# 20011-1, goat (non-immune) IgG, purified, suitable for ELISA, Western, IHC as –ve control

Human Ngr-Fc protein Western blot +ve control is (**Cat #NGR12-C**) is supplied in Laemmeli SDS-PAGE sample buffer (reduced). Load 5-10 ul/lane to visualize it with antibodies (Cat #NGR12-A). Aliquot and freeze in suitable aliquots. Heat once prior to electrophoresis. Do not freeze and thaw. This preparation of Ngr-Fc protein is denatured and not biologically active. It is also not suitable for coating on ELISA plates or applications other than Western. ADI supplies another preparation of Ngr-Fc that is biologically active (cat #NGR15-R-50).

Form & Storage of Antibodies/Control

Storage

Short-term: unopened, undiluted vials for less than a week at 4oC.

Long-term: at –20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20oC or below.

Shipping: 4oC for solutions and room temp for powder.

Recommended Usage

Western Blotting (1-2 ug/ml for affinity pure using Chemiluminescence technique). Ngr-Fc ~100 kda.

ELISA (0.1-1 ug/ml antibody) detection of Ngr-Fc is ~0.1 ug/well.

Histochemistry: Not tested**.** We recommend the use of 2-10 ug/ml of affinity pure IgG.

Specificity & Cross-reactivity

Anti-human NgR-Fc has minimal crossreactivity with mouse/rat Ngr. We recommend using anti-mouse Ngr (cat #NGR13-A) for mouse/rat Ngr. Recombinant mouse Ngr-Fc protein (Cat #NGR13-C) is also available for control studies.

General References: 1. Fournier AE et al (2001) Nature 409, 341-346, Chen MS et al (2000) Nature 403, 434-439; GrandPre T et al (2000) Nature 403; 439-444; Goldberg JL and Barres BA (2000) Nature 403; 369-370; Prinjha R et al (2000) Nature 403, 383-384; Tessier-Lavigne M and Goodman CS (2000) Science 287, 813-814; Nagase T et al (1998) DNA Res. 5, 355-364

*This product is for In vitro research use only.

Related material available from ADI

Anti-Nogo A, -B, -C, Recombinant Ngr, NEP1-40

Ngr12-A-C 70829J

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