ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Neprilysin-Like Protease-γ (NEPL-γ) Antibodies

Cat. # NEPLG31-S	Rabbit Anti-Mouse NEPL-γ antiserum	**SIZE**: 100 ul
Cat. # NEPLG31-A	Rabbit Anti-Mouse NEPL-γ IgG (aff pure)	**SIZE**: 100 ug
Cat. # NEPLG31-P	Mouse NEPL-γ Control/blocking peptide	**SIZE**: 100 ug

The amyloid β-peptide (**Aβ**) of 39 to 43 amino acids is constitutively produced in brain upon proteolysis of the β-amylod precursor protein (**APP**) and exists as fragments of 40, 42 and 43 amino acids ($A\beta_{40}$, $A\beta_{42}$, $A\beta_{43}$). In the young and healthy humans, the secreted Aβ is rapidly catabolized before it can be deposited in the brain. However, upon aging or the onset of familial **Alzheimer's disease**, alterations in either synthesis or degradation/clearance of Aβ may contribute to amyloid depositions in the brain. Aβ carries cleavage sites for a number of *in vivo* and *in vitro* proteases like cathepsin D and M-13 metalloproteases. The M-13 family comprises several zinc-dependent metalloproteases like **DINE**, **PHEX**, **KELL**, **ECE**, **XCE**, **neprilysin** (**NEP**) and neprilysin-like proteases (**NEPLs**). The NEPLs (**NEPL-α, NEPL-β, NEPL-γ**) arise from the alternative splicing of a single NEPL gene and are zinc dependent metalloproteases with ~54 % homology to NEP.

NEPL-γ is a **115 kDa (**779 aa) with Zinc binding HEXXH motif in its extracellular domain. NEPL-γ appears to have a different conformation and substrate specificity than those of NEPL-α. In comparison to NEPL-α, NEPL-γ has an additional sequence of 37aa (311-347) near the center of the polypeptide, which possibly inhibits its activity by interfering with proper folding and interaction with the substrate. Unlike NEP, NEPL-γ has no proteolytic activity to either synthetic or Aβ peptides and does not appear to be a possible *in vivo* peptidase for endogenous Aβ. Proteolytic activation of endopeptidase activity of NEPL-γ by a partial/complete removal of the unique region (311-347aa) in its sequence has been proposed.

Source of Antigen and Antibodies

Antigen	A 15-aa peptide sequence **(gene accession #NP07365.1)** (**designated NEPLG31-P or control peptide**), mapping at the **extracellular domain** (within the unique 37-aa insert) of **mouse NEPL-gamma** (1)
Ab Host/type	Rabbit, Polyclonal antiserum # **NEPLG31**-S and IgG, purified over antigen-agarose (Cat # **NEPLG31**-A)
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available).
-ve control IgG	Cat # 20009-1, Rabbit (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)
100ul solution lyophilized powder
Supplied 0.05% azide, **Reconstitute** powder in 100 ul PBS

Affinity pure IgG
100 ug/100ul solution lyophilized powder
Supplied in **Buffer**: PBS+0.1% BSA
Reconstitute powder in PBS at 1mg/ml

Control/blocking peptide
100 ug/100 ul solution lyophilized powder
Supplied in Buffer: PBS pH 7.5,
Reconstitute powder in PBS at 1 mg/ml.

Storage
Short-term: unopened, undiluted liquid vials at -20OC and powder at 4oC or -20oC..
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 (1:1K-5K for neat serum and 1-10 ug/ml for affinity pure using Chemiluminescence technique).

ELISA (1:10K-1:100K; using 50-100 ng of control peptide/well).

Histochemistry & Immunofluorescence: not tested. We recommend the use of affinity pure antibody at 2-20 ug/ml.

Specificity & Cross-reactivity

The NEPLG31-P antigenic/control peptide is conserved in mouse (100%) NEPL-γ. No significant sequence homology of NEPLG31-P is seen with other proteins. Antibody reactivity in various species is not known. The NEPLG31-P Control peptide, because of its low mol. Wt (<3 kDa), is not suitable for Western. It should be used for ELISA or antibody blocking experiments (use 5-10 ug control peptide per 1 ug of aff pure IgG or 1 ul antiserum) to confirm antibody specificity (see detailed protocol see detailed protocol at the web site).).

General References: (1) Shirotani K et al (2001) JBC 276, 21895-21901; Iwata et al. (2001) Science 292, 1550; Ikeda et al. (1999) J. Biol. Chem. 274, 32469; Boileau et al. (2001) Biochem. J 355, 107; Kiryu-Seo et al. (2000) PNAS 97, 4345.

**This product is for In vitro research use only.*

Related materials available from ADI

Antibodies: NEP, NEP-alpha, -beta, -gamma, DINE, PHEX.

NEPLG31-S-A-P 71212A

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