ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

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

Cat # NEPLB21-S	Rabbit Anti-Mouse NEPL-β antiserum	**SIZE**: 100 ul
Cat # NEPLB21-A	Rabbit Anti-Mouse NEPL-β IgG (Affinity pure)	**SIZE**: 100 ug
Cat # NEPLB21-P	Mouse NEPL-β 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-β (variously called SEP/NL1/NEPII) is type II transmembrane enzyme containing a single polypeptide chain of 765aa (~110 KDa) with cytosolic and transmembrane domains and a large extracellular C-terminal core containing the peptidase active site. The aa sequence is 65.1 % identical to mouse NEP. Like NEP, NEP-β also contains 12 cysteine residues, 10 of which are conserved in all members of the family. There are two splice variants of NEP-β; a secreted isoform of 126 KDa containing a 23 aa secretion signal sequence and a membrane associated isoform of 110 kDa. The secreted isoform is much more glycosylated than the membrane isoform. Testis is the only tissue where the soluble/secreted isoform is predominant. Unlike NEP, NEPL-β has no proteolytic activity to Aβ; however both enzymes can cleave Leu_5-enkephalin.

FUNCTION: Metalloprotease involved in sperm function, possibly by modulating the processes of fertilization and early embryonic development.
SUBCELLULAR LOCATION: Membrane; Single-pass type II membrane protein. Secreted. Note=A secreted form produced by proteollytic cleavage also exists.
SIMILARITY: Belongs to the peptidase M13 family [view classification].

Protein name Membrane metallo-endopeptidase-like 1
Synonyms EC 3.4.24.11,Neprilysin-2, Neprilysin II, NL2
NEPII, NEP2(m), Neprilysin-like peptidase, NEPLP,Neprilysin-like 1
NL-1, Soluble secreted endopeptidase
Gene name Name: Mmel1 ; Synonyms: Nep2, Nl1, Sep

Source of Antigen and Antibodies

Antigen	A 13-aa peptide sequence **(gene accession#Q9JLI3)** (**designated NEPLB21-P or control peptide**), conjugated to KLH Epitope location ~ N-terminus
Ab Host/type	Rabbit, Polyclonal aff pure IgG (cat # NEPLB21-A) purified over the antigen column
2-ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available
-ve control	# 20009-1, Rabbit (non-immune) IgG, purified, suitable for ELISA, Western, IHC as –ve control

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)
☐ 100 ul ☐ Solution ☐ Lyophilized powder
Supplied in Buffer: 0.1% azide
Reconstitute powder in 100 ul PBS

Affinity pure IgG

☐ 100 ug/100 ul ☐ Solution ☐ Lyophilized powder
Supplied in **Buffer**: PBS + 0.1% BSA
Reconstitute powder in PBS at 1 mg/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 -20°C and powder at 4°C or -20°C..

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

Stability: 6-12 months at -20°C or below.
Shipping: 4°C 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).NOT TESTED

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

Mouse NEPLB21-P antigenic/control peptide sequence is 76% conserved in rat NEPL-β. No significant sequence homology of NEPLB21-P is seen with NEP or other NEPLs. Antibody reactivity in various species is not known. 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 at: www.4adi.com\data\abblock.html).

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.

NEPLB21-S-A-P rev. 210112AC

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