

Product Specification Sheet

**Neprilysin-Like Protease- $\gamma$  (NEPL- $\gamma$ ) Antibodies**

Cat. # NEPLG31-S	Rabbit Anti-Mouse NEPL- $\gamma$ antiserum	<b>SIZE:</b> 100 ul
Cat. # NEPLG31-A	Rabbit Anti-Mouse NEPL- $\gamma$ IgG (aff pure)	<b>SIZE:</b> 100 ug
Cat. # NEPLG31-P	Mouse NEPL- $\gamma$ Control/blocking peptide	<b>SIZE:</b> 100 ug

The amyloid  $\beta$ -peptide (**A $\beta$** ) of 39 to 43 amino acids is constitutively produced in brain upon proteolysis of the  $\beta$ -amyloid precursor protein (**APP**) and exists as fragments of 40, 42 and 43 amino acids (A $\beta$ <sub>40</sub>, A $\beta$ <sub>42</sub>, A $\beta$ <sub>43</sub>). In the young and healthy humans, the secreted A $\beta$  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 A $\beta$  may contribute to amyloid depositions in the brain. A $\beta$  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- $\alpha$** , **NEPL- $\beta$** , **NEPL- $\gamma$** ) arise from the alternative splicing of a single NEPL gene and are zinc dependent metalloproteases with ~54 % homology to NEP.

**NEPL- $\gamma$**  is a **115 kDa** (779 aa) with Zinc binding HEXXH motif in its extracellular domain. NEPL- $\gamma$  appears to have a different conformation and substrate specificity than those of NEPL- $\alpha$ . In comparison to NEPL- $\alpha$ , NEPL- $\gamma$  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- $\gamma$  has no proteolytic activity to either synthetic or A $\beta$  peptides and does not appear to be a possible *in vivo* peptidase for endogenous A $\beta$ . Proteolytic activation of endopeptidase activity of NEPL- $\gamma$  by a partial/complete removal of the unique region (311-347aa) in its sequence has been proposed.

**Source of Antigen and Antibodies**

<b>Antigen</b>	A 15-aa peptide sequence ( <b>gene accession #NP07365.1</b> ) ( <b>designated NEPLG31-P or control peptide</b> ), mapping at the <b>extracellular domain</b> (within the unique 37-aa insert) of <b>mouse NEPL-gamma (1)</b>
<b>Ab Host/type</b>	Rabbit, Polyclonal antiserum # <b>NEPLG31-S</b> and IgG, purified over antigen-agarose (Cat # <b>NEPLG31-A</b> )
<b>2-Ab</b>	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available).
<b>-ve control IgG</b>	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 -200C 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- $\gamma$ . 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) Shirovani 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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