

Product datasheet for **AM26395PU-L**

Amyloid beta (N-term) Mouse Monoclonal Antibody [Clone ID: NT 3F5]

Product data:

Product Type:	Primary Antibodies
Clone Name:	NT 3F5
Applications:	IHC
Recommended Dilution:	Immunohistochemistry Frozen Sections (See protocol below).
Reactivity:	Human
Host:	Mouse
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	Full length Human beta amyloid peptide 42
Specificity:	This antibody recognizes the N-terminal sequence of beta Amyloid peptides.
Formulation:	0.01M PBS, pH7.2 State: Aff - Purified State: Lyophilized purified IgG fraction
Reconstitution Method:	Double distilled water is recommended and to adjust the final concentration to 1.0 mg/ml.
Purification:	Affinity Chromatography on Protein G
Conjugation:	Unconjugated
Storage:	Store (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.



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Background:

Beta amyloid, often abbreviated as A-beta, is a protein that builds up in the brains of persons with Alzheimer's disease, collecting in clumps called plaques or senile plaques. While some researchers question whether beta amyloid is the cause of the dementia, most agree that it is involved in the disruption of thinking that is a hallmark of the disease. In some cases of familial Alzheimer's disease, mutations in genes for the proteins called the presenilins lead to increased production of amyloid. Researchers have been looking at how presenilin-1 in particular contributes to the excess buildup of beta amyloid. Presenilin-1 apparently acts to increase the activity of gamma-secretase, an enzyme that changes a normal protein (amyloid precursor protein or APP) into beta amyloid itself. Furthermore, presenilin-1 might be gamma-secretase.

Note:

Protocol:

Immunohistochemical Detection of AD tissues using Anti-A β 42 antibodies**Methods:**Preparation of Tissue Sections

□1□ Cut cryostat tissue sections 4-6 μ m thick and mount on poly-L-Lysine coated or superfrost microscope slides.

□2□ Dry the tissue sections under a cold blow dryer for at least 30 min.

□3□ Use the tissue sections immediately or store them in a sealed box at -20°C or -80°C.

Immunohistochemistry:

(1) Fix the tissue sections for 10 min at room temperature in 4% paraformaldehyde diluted in PBS containing 0.5% glucose.

(2) Rinse the sections in PBS-S. It is convenient to place the sections in a coplin jar during all washing steps. During incubation steps, place the slides horizontally in a moist chamber.

(3) Inhibit endogenous peroxidase activity in the tissue sections by incubating in 1% H₂O₂/0.02% Na₃N in PBS-S for 30 min (15 ml PBS-S+0.5 ml 30% H₂O₂). Rinse the sections briefly in PBS-S.

(4) Incubate the sections in blocking-buffer for 10 min to block nonspecific binding sites.

(5) Remove excess of blocking-buffer from the slides with a tissue and incubate the sections with the primary antibody diluted 2-5 μ g/ml in PBS-BSA-S overnight at 4°C.

(6) Rinse the sections in PBS-S for 3 \times 5 min.

(7) Remove excess of PBS-S with a tissue and apply the HRP-secondary antibody diluted 2-5 μ g/ml in PBS-BSA-S and incubate for 30-60 min at RT.

(8) Rinse the sections in PBS-S for 3 \times 5 min.

(9) Incubate the sections in freshly prepared DAB substrate solution for 3-5 min. A brown reaction product will be deposited at the site of primary antibody binding (2 ml DAB+15 μ l 3% H₂O₂)

(10) Rinse the sections in running tap water for 5 min.

(11) Counterstain lightly with hematoxylin for 1 min.

(12) Dehydrate the sections by incubation at increasing concentrations of ethanol (70,96,100%), finally clear with xylene, and mount in Depex.

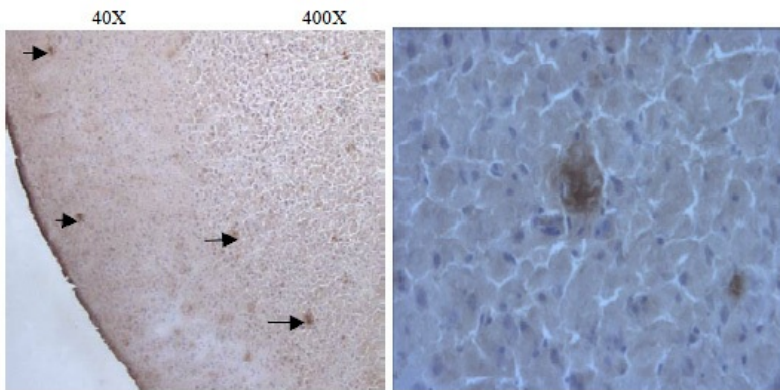
Materials:

(1) Paraformaldehyde-fixed cryostat tissue sections.

(2) Phosphate-buffered saline (PBS, 0.01M, pH7.4).

- (3) PBS-S: 200 ml PBS+0.8 ml 25% glucose+0.6 ml TritonX-100.
- (4) PBS-BSA-S: 1.8 ml PBS-S+200 ul 10% BSA
- (5) 1% hydrogen peroxide in PBS-S containing 0.02% NaN₃
- (6) Blocking-buffer: Normal serum of the species in which the secondary antibody was raised is used diluted to 2-5% in PBS-BSA-S to block nonspecific binding sites□PBS-BSA-S+5% serum□
- (7) Primary antibody diluted in PBS-BSA-S.
- (8) HRP-secondary antibody diluted in PBS-BSA-S.
- (9) Purchase DAB from DAKO, operating according to ako Elisa Kit.
- (10) Hematoxylin.
- (11) 70%, 96 %and 100% ethanol.
- (12) Xylene.
- (13) Cover slips.
- (14) Depex-mounting medium (BDH Laboratory Supplies, Poole, England).

Product images:



NT 3F5
(1:100)

Immunohistochemistry experiment using NT-3F5 (1/100) on the brain tissue from transgenic Alzheimer's disease mouse model. The plaques showed strong positive signal and the background was clear.