NEUROMICS

Amyloid Precursor Protein 228 (APP 228) Data Sheet

Catalog Number:	MO20015	**Host:**	Mouse
Ig Class:	IgM Clone: 3G12	**Species Reactivity:**	Human
Immunogen Sequence:	Prokaryotic recombinant fusion protein corresponding to the extracellular protein of APP between the Kunitz protease inhibitor domain and the beta amyloid region.	**Format:**	Liquid- tissue culture supernatant containing 15mM sodium azide.

Applications: Immunohistochemistry: 1:25 - 1:50. Tested on Paraffin Embedded Tissue Only.
Western Blotting*

Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.

Storage: Antibody can be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. *Avoid repeated freeze-thaw cycles.*

References: Rita Costa, Frederico Ferreira-da-Silva, Maria J. Saraiva, and Isabel Cardos. Transthyretin Protects against A-Beta Peptide Toxicity by Proteolytic Cleavage of the Peptide: A Mechanism Sensitive to the Kunitz Protease Inhibitor. PLoS ONE. 2008; 3(8): e2899. Published online 2008 August 6. doi: 10.1371/journal.pone.0002899.

Application Notes

Specificity:
Some deposited isoforms of human amyloid precursor protein (APP) which are present in late stage neurofibrillary tangle-bearing neurons, neuritic processes surrounding senile plaques and neuropil threads in grey matter of Alzheimer's diseased brain but not with isoforms of APP found in large pyramidal cells, smaller neurons and astrocytes. No crossreaction with APP-like proteins is evident.

Staining Pattern:
Neurofibrillary tangles and senile plaques. For paraffin embedded tissue, use 1mM EDTA (pH8.0) unmasking solution combined with the high temperature antigen for 5 minutes.

*See Rita Costa, Frederico Ferreira-da-Silva, Maria J. Saraiva, and Isabel Cardoso. Transthyretin Protects against A-Beta Peptide Toxicity by Proteolytic Cleavage of the Peptide: A Mechanism Sensitive to the Kunitz Protease Inhibitor. PLoS ONE. 2008; 3(8): e2899. Published online 2008 August 6. doi: 10.1371/journal.pone.0002899-Human, recombinant or sera, TTR (15 µg) and A-Beta peptide (1–42) (2 µg) were incubated in 20 µl 50 mM Tris, pH 7.5, at 37°C for different periods of time, and then reactions were applied onto a 15% SDS-PAGE gel and visualized by coomassie blue staining. Alternatively, TTR incubated with A-Beta (1–42) and A-Beta alone, were run on a SDS-PAGE gel, transferred to nitrocellulose membranes, and A-Beta immunodetected using an anti-A-Beta antibody (BAM-10, Sigma). In another set of experiments, A-Beta was incubated for 6 hours at 37°C for aggregation purposes and then subsequently incubated with TTR. Results were visualized by immunoblot, as described above.

To evaluate possible inhibitors of A-Beta proteolysis by TTR, recombinant TTR (15 µg) was pre-incubated for 30 minutes at 37°C, either with 1 mM pefabloc, 0.33 µM αAPP of peptide encompassing aminoacids 18–688, containing the KPI domain (Neuromics) or with 0.33 µM αAPP peptide derived from the APP isoform 695 (without the KPI domain) (Sigma), both formed by a α-secretase cleavage. Then, A-Beta was added (2 µg) in a final volume of 20 µl, and further incubated for 6 hours at 37°C. Results were observed by immunoblot as described. Experiments were repeated at least twice.

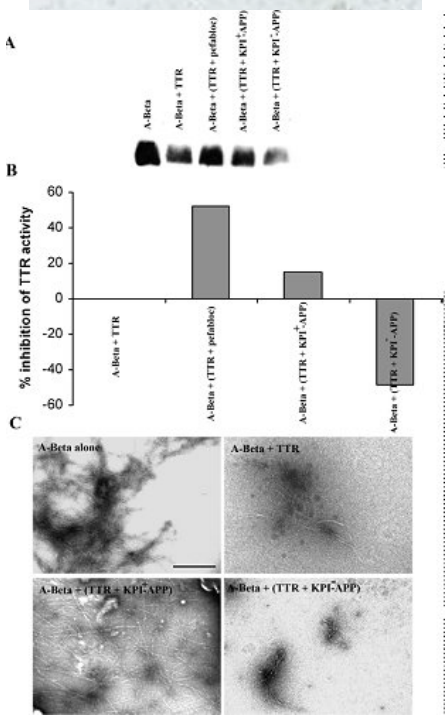
FOR RESEARCH USE ONLY

Neuromics Antibodies • 5325 West 74th Street, Suite 8 • Edina, MN 55439
phone 866-350-1500 • fax 612-677-3976 • e-mail: pshuster@neuromics.com

Description/Data:
Alzheimer's disease, the most common cause of dementia in the elderly, exists in both familial and sporadic forms. Genetic studies have identified three genes; beta-amyloid precursor protein (APP), Presenilin 1 and Presenilin 2 which, when mutated, can cause familial forms of Alzheimer's disease. APP and APP-like proteins are transmembrane glycoproteins with a similar modular domain structure.
APP-228 has been raised to the extracellular portion of APP between the Kunitz protease inhibitor domain and the beta amyloid region. This region shows the least homology with the APP-like proteins. APP-228 and does not cross-react with APP-like proteins. APP reacts with large pyramidal cells as well as smaller neurons, astrocytes and microglia. APP 228 reacts with late-stage neurofibrillary tangle-bearing neurons, neuritic processes surrounding senile plaques and neuropil threads in gray matter of Alzheimer's disease brain. Unmasking in 1mM EDTA (pH8.0) in a pressure cooker may be required for up to 5 minutes in order for this APP-228 to work optimally.

Image: Human cortex, Alzheimer's disease: immunohistochemical staining using amyloid precursor protein-228. Note intense staining of neurofibrillary tangles and senile plaques. Paraffin section.

A-Beta proteolysis by TTR is KPI-sensitive.
A- A-Beta incubated with TTR (A-Beta+TTR) shows a weaker A-Beta monomer band as compared to A-Beta alone (A-Beta), indicative of proteolysis, as analyzed by SDS-PAGE electrophoresis followed by western blot. Pre-incubation of TTR with pefabloc (A-Beta+(TTR+pefabloc)) and with an αAPP peptide containing the KPI domain (A-Beta+(TTR+KPI+−APP)) inhibits TTR proteolytic activity, whereas the αAPP peptide without the KPI domain (A-Beta+(TTR+KPI−−APP)) facilitates proteolysis. B- % of inhibition of TTR proteolysis by quantification of band intensity in A. C- Ultrastructural analysis by TEM of preparations incubated for 15 hours, as described in Materials and Methods. TTR inhibited A-Beta aggregation as compared with A-Beta incubated alone (upper panels). Pre-incubation of TTR with αAPP peptide containing the KPI domain (A-Beta+(TTR+KPI+−APP)) abrogated TTR ability to avoid A-Beta aggregation, whereas αAPP lacking the KPI domain (A-Beta+(TTR+KPI−−APP)) did not affected TTR activity (lower panels). Scale bar=500 nm. PLoS ONE. 2008; 3(8): e2899. Published online 2008 August 6. doi: 10.1371/journal.pone.0002899.



FOR RESEARCH USE ONLY

Neuromics Antibodies • 5325 West 74th Street, Suite 8 • Edina, MN 55439
phone 866-350-1500 • fax 612-677-3976 • e-mail: pshuster@neuromics.com