rPeptide offers a wide range of reagents for research purposes in the study of neurodegenerative diseases such as Parkinson’s and Alzheimer’s. These include the 6 forms of Tau protein made via recombinant expression in E.coli with no affinity tags.

The Tau isoforms vary in size from 352 to 441 amino acids (36.8 to 45.9 kDa), and differ from one another in having three (3R) or four (4R) microtubule binding repeats (R) of 31-32 amino acids, with four of the six isoforms contain between one (1N) and two (2N) amino terminal inserts (N) of 29 amino acids each1. Tau functions by promoting the assembly and maintaining the structure of microtubules in neuronal cells2,3,4.

rPeptide’s Tau proteins are made to a purity of 90% or greater and packaged in 50µg and 100µg quantities.

rPeptide is now offering a new monoclonal antibody Tau 1A6, which reacts with all six isoforms of Tau. This IgM antibody provides the researcher an additional reagent in their investigation on neurodegenerative diseases.

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Product</th>
<th>Variant</th>
<th>Exon 2</th>
<th>Exon 3</th>
<th>Exon 10</th>
<th>AA</th>
<th>Mass (kDa)</th>
<th>Expressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-1001-1</td>
<td>Tau-441</td>
<td>2N4R</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>441</td>
<td>45.9</td>
<td>Adult</td>
</tr>
<tr>
<td>T-1002-1</td>
<td>Tau-410</td>
<td>2N3R</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>410</td>
<td>42.6</td>
<td>Adult</td>
</tr>
<tr>
<td>T-1003-1</td>
<td>Tau-412</td>
<td>1N4R</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>412</td>
<td>42.9</td>
<td>Adult</td>
</tr>
<tr>
<td>T-1004-1</td>
<td>Tau-381</td>
<td>1N3R</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>381</td>
<td>39.7</td>
<td>Adult</td>
</tr>
<tr>
<td>T-1005-1</td>
<td>Tau-383</td>
<td>0N4R</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>383</td>
<td>40</td>
<td>Adult</td>
</tr>
<tr>
<td>T-1006-1</td>
<td>Tau-352</td>
<td>0N3R</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>352</td>
<td>36.8</td>
<td>Fetal</td>
</tr>
</tbody>
</table>

References:
2. Avila J. et. al., 2004, Physiol Rev. 84, 361.

In addition to the highest quality products, rPeptide also offers over 16 years of Parkinson’s and Alzheimer’s related experience and knowledge that allow us to assist you with any of your research needs. rPeptide can also provide larger quantity packages of all of our products, and all the reagents can be offered as a kit or as individual components.

rPeptide’s goal is to provide you with the highest quality products and exceptional customer service.
Now Available
Tau 4G11 Antibody

rPeptide offers a wide range of reagents for research purposes in the study of neurodegenerative diseases such as Parkinson’s and Alzheimer’s. These include the 6 forms of Tau protein made via recombinant expression in E.coli with no affinity tags.

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Product</th>
<th>Variant</th>
<th>Exon 2</th>
<th>Exon 3</th>
<th>Exon 10</th>
<th>AA</th>
<th>Mass (kDa)</th>
<th>Expressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-1001-1</td>
<td>Tau-441</td>
<td>2N4R</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>441</td>
<td>45.9</td>
<td>Adult</td>
</tr>
<tr>
<td>T-1002-1</td>
<td>Tau-410</td>
<td>2N3R</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>410</td>
<td>42.6</td>
<td>Adult</td>
</tr>
<tr>
<td>T-1003-1</td>
<td>Tau-412</td>
<td>3N4R</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>412</td>
<td>42.9</td>
<td>Adult</td>
</tr>
<tr>
<td>T-1004-1</td>
<td>Tau-381</td>
<td>1N3R</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>381</td>
<td>39.7</td>
<td>Adult</td>
</tr>
<tr>
<td>T-1005-1</td>
<td>Tau-383</td>
<td>0N4R</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>383</td>
<td>40</td>
<td>Adult</td>
</tr>
<tr>
<td>T-1006-1</td>
<td>Tau-352</td>
<td>0N3R</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>352</td>
<td>36.8</td>
<td>Fetal</td>
</tr>
</tbody>
</table>

The Tau isoforms vary in size from 352 to 441 amino acids (36.8 to 45.9 kDa), and differ from one another in having three (3R) or four (4R) microtubule binding repeats (R) of 31-32 amino acids, with four of the six isoforms contain between one (1N) and two (2N) amino terminal inserts (N) of 29 amino acids each1. Tau functions by promoting the assembly and maintaining the structure of microtubules in neuronal cells2,3,4.

rPeptide’s Tau proteins are made to a purity of 90% or greater and packaged in 50µg and 100µg quantities.

rPeptide is now offering a new monoclonal antibody, Tau-4G11 that binds to isoforms 381, 410, 412 and 441 all of which contain at least one amino terminal repeat. This lgA antibody provides researchers with a new tool to analyze differential expression of the six tau isoforms.

References:
2. Avila J. et al., 2004, Physiol Rev. 84, 361.

In addition to the highest quality products, rPeptide also offers over 16 years of Parkinson’s and Alzheimer’s related experience and knowledge that allow us to assist you with any of your research needs. rPeptide can also provide larger quantity packages of all of our products, and all the reagents can be offered as a kit or as individual components.

rPeptide’s goal is to provide you with the highest quality products and exceptional customer service.
Recombinant Beta Amyloid Peptides

Benefits You Receive

- Batch to batch consistency of physical, chemical and biological properties.
- Purity is consistently >97%
- There is no oxidation of the 35Met. A mutant 35Met-Valine, is available to study the effect of oxidation of methionine.
- No chemical modifications, such as racemerization.
- Uniformly full length peptides, no n-1, n-2 subspecies in each lot
- Ability to economically and uniformly label with $^{15}$N, $^{13}$C and $^{15}$N+$^{13}$C
- Recombinant Beta Amyloid sold as “net” peptide content rather than ‘total’ content, which includes 20%-40% salt.

rPeptide’s recombinant beta-amyloid peptides are prepared as a very soluble (proprietary) fusion, to significantly reduce the non-specific binding in an impure prep. This soluble fusion is purified to >90% purity and then cleaved to give the final beta-amyloid peptides, which are further purified to >97% purity. As a result, rPeptide’s recombinant beta-amyloids gives high batch to batch reproducibility that is confirmed by analytical HPLC and mass spectroscopy experiments.

References

AD patients seem to express increased alpha 1-Antichymotrypsin (AACT) index, suggesting an intrathecal production of AACT. Indeed, AACT is a major component of the amyloid plaques found in the brains of AD patients. In Parkinson’s disease (PD), AACT gene has been suggested as a susceptibility factor that might be related to the onset of PD.\(^1\)\(^2\)

Myeloperoxidase (MPO) was found to be a key oxidant-producing enzyme during inflammation and it appeared to be upregulated in the ventral midbrain of human PD. It has been suggested that inhibitors of MPO “may provide a protective benefit in PD”\(^3\). Although unexpected, myeloperoxidase expression was found in neurons and it increased in brain tissue showing AD neuropathology. Thus it is possible that MPO contributes to the oxidative stress implicated in the pathogenesis of the neurodegenerative disorder.

Plasmin

Isolated from human plasma

The plasmin system is involved in the degradation of Abeta peptides\(^7\). Brain plasmin enhances APP alpha-cleavage and Abeta degradation and is reduced in Alzheimer's disease brains\(^7\)\(^8\).

Alpha 2 Macroglobulin

Prepared from plasma

Alpha 2 Macroglobulin (A2M) seems to play a role in the development of sporadic AD. A2M-D allele was observed as a weak AD protective factor, and the possible interaction of APOE-epsilon 4 and A2M-G alleles may have cause an “increase AD risk in Mainland Han Chinese”\(^6\).

### Alpha 1 Antichymotrypsin

**Prepared from human plasma**

Like alpha 1-antichymotrypsin alpha 1-antichymotrypsin might be functionally involved in the pathogenesis of the lesions of Alzheimer's disease. Both serine protease inhibitors are found in neurofibrillary tangles and senile plaques. The major cell producing alpha 1-antitrypsin localization in seems to be the astrocytes, which are involved in both lesions.\(^5\)

### Alpha 1 Antitrypsin

**An acute phase plasma protein**

Like alpha 1-antichymotrypsin, alpha 1-antitrypsin might be functionally involved in the pathogenesis of the lesions of Alzheimer's disease. Both serine protease inhibitors are found in neurofibrillary tangles and senile plaques. The major cell producing alpha 1-antitrypsin localization in seems to be the astrocytes, which are involved in both lesions.\(^5\)
Plasminogen
Isolated from human plasma

Plasminogen is the precursor of the serine protease plasmin which is involved in fibrinolysis. The Tissue Plasminogen Activator-Plasminogen Proteolytic Cascade Accelerates Amyloid-β (Aβ) Degradation and Inhibits Aβ induced Neurodegeneration9.

Transferrin
Isolated from human plasma

Transferrin is a glycosylated metal-carrying serum protein. Transferrin, the transport protein of iron, has been implicated as playing a role in AD and PD by regulating the iron concentrations in the brain12,13.

Haptoglobin
Isolated from plasma

Particular fragments of Haptoglobin are found in the circulation of patients suffering from Alzheimer's dementia. Haptoglobin fragments have been use in the efforts to better diagnose Alzheimer's disease and determine risk-assessment, disease progression, disease state, therapeutic avenues, among other pharmaceutical agents deemed to be efficacious in treating the disease10,11.

Chymotrypsin
Isolated from human plasma

Chymotrypsin-like proteasome activities were found to increase in cells treated with 10–100 µM 6-OHDA, while “higher doses caused a marked decline”14. Such harsh oxidative attacks might cause failure of the ubiquitin-proteasome system (UPS) which leads to protein aggregation and cell death. “Mutations in familial PD have been associated with the failure of protein degradation through UPS”14.

References

11European Patent EP1495331

*Not for human use, for research purposes only.
Calmodulin (CaM) is a ubiquitous, calcium-binding protein that binds and regulates a multitude of protein targets, many of which are involved in the Alzheimer’s and the Parkinson’s pathways. CaM has a molecular weight of about 17kDa, containing 148 amino acids, and pI of 3.9. CaM is characterized by two domains, connected by an alpha-helix chain. Each domain has the capacity to bind two calcium ions. Binding Ca^{2+} ions causes a conformational change in CaM, making it available for interaction with target proteins. Hence, CaM functions as an intracellular calcium ion bridge to mediate cellular reactions and responds appropriately to calcium ion concentration. In Alzheimer’s disease (AD), irregular calcium homeostasis seems to trigger CaM and its binding proteins, to enhance plaque formation and neurofibrillary degeneration, which results in cell death.

The increased cytosolic levels of Ca^{2+} in AD neurons promotes CaM binding and regulation of available Ca^{2+}/CaM-dependent CaM-binding proteins, associated with amyloid beta (Ab) formation. In addition, the increased level of Ca^{2+} triggers Calmodulin to activate calcium/CaM-dependent kinase II and precede neurofibrillary tangle formation. In Parkinson’s disease (PD), Calmodulin has been found to interact, in a calcium dependent manner, with Alpha-Synuclein, which is associated with the progression of PD. CaM was identified as one of the synuclein-interacting proteins that regulate synuclein conformation.

References


*Not for human use, for research purposes only.*
α-Synuclein is a 14 kD (140 amino acids) acidic presynaptic protein. It is a major component of Parkinson’s disease aggregates and is implicated in the pathogenesis of Parkinson’s disease and related neurodegenerative disorders. α-Synuclein accumulates in the brains of sporadic Parkinson’s disease patients as a major component of Lewy bodies, which are intraneuronal cytoplasmic inclusions characteristic of Parkinson’s disease. α-Synuclein appears to associate with other proteins that aggregate and is found in β-Amyloid plaques and neuritic tangles in Alzheimer’s disease.

References
7 Lashuel, H.A., et. al., 2002, J Mol Biol. 322, 1089
12 Bruening, W. et al. 2000, Cancer **88**, 2154

*Not for human use, for research purposes only.*
**Mutant Tau Proteins**

**Human Recombinant E. coli**

**No His Tag**

*Tau is a family of six isoforms, derived from a single gene by alternative mRNA splicing*\(^1\). *They vary in size from 352 to 441 amino acids (36.8 to 45.9 kDa), and differ from one another in having three or four microtubule binding repeats (R) of 31-32 amino acids each, and two, one or none amino terminal inserts (N) of 29 amino acids each*\(^2\).*

Tau promotes the assembly and maintains the structure of microtubules in neuronal cells\(^3,4,5\). While the fetal brain contains a single isoform of tau (Tau-352) the adult brain has several isoforms. Tau is both phosphorylated and O-GlcNAcylated\(^6\). The normal brain tau contains 2-3 moles of phosphate/mole of the protein. In Alzheimer disease tau is hyperphosphorylated, containing 3-4-fold more phosphate/mole of the protein than the normal tau\(^7,8\) and is the major protein subunit of paired helical filaments (PHF) that form the neurofibrillary tangles (NFT). NFT accumulation correlates with the clinical progression of Alzheimer's disease.

The discovery of close to 20 different mutations in the gene encoding the microtubule-associated protein tau in frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17) has shown that dysfunction of tau protein causes neurodegeneration and dementia\(^9\).

**References**

1. Himmler, et. al., 1989, Mol Cell Biol. 9, 1381
3. Avila J. et. al., 2004, Physiol Rev. 84, 361.
8. Kopke, et. al., 1993, J. Biol. Chem. 268, 2437
Tau SDS-PAGE Samples

Tau 441 G272V- (>90%) (no His-Tags) SDS-PAGE Purity Profile

Tau 441 R406W- (>90%) (no His-Tags) SDS-PAGE Purity Profile

Tau 441 P301L- (>90%) (no His-Tags) SDS-PAGE Purity Profile

Tau 441 V337M- (>90%) (no His-Tags) SDS-PAGE Purity Profile

Tau 441 V248L- (>90%) (no His-Tags) SDS-PAGE Purity Profile

References

1. Himmler, et. al., 1989, Mol Cell Biol. 9, 1381
3. Avila J. et. al., 2004, Physiol Rev. 84, 361.
8. Kopke, et. al., 1993, J. Biol. Chem. 268, 2437

*Not for human use, for research purposes only.*
All Six Isoforms of Tau

Tau is a family of major neuronal microtubule associated proteins that are found in the neurofibrillary tangles (NFT) in Alzheimer’s disease. Tau promotes the assembly and maintains the structure of microtubules in neuronal cells\(^1,2,3\). The Tau proteins are derived from alternative mRNA splice variants that originate from a single gene and result in mature proteins that vary in size from 352 to 441 amino acids (36.8 to 45.9 kDa).

There are six Tau isoforms, that differ from one another in having three or four microtubule binding repeats (R) of 31-32 amino acids each, and two, one or none amino terminal inserts (N) of 29 amino acids each\(^4\). While the fetal brain contains a single isoform of tau (Tau-352) the adult brain has several isoforms all derived from a single gene by alternative mRNA splicing\(^5\).

**Electrophoresis.** 10μl of tau protein marker (mix of six human tau isoforms) was run on a 4-20% gradient gel to verify purity.

***References***

1. Himmler, et. al., 1989, Mol Cell Biol. 9, 1381
3. Avila J. et. al., 2004, Physiol Rev. 84, 361.

*Not for human use, for research purposes only.*
rPeptide is happy to announce the recent expansion of our facilities and services offered. We now offer custom protein purifications on a much wider scale, which ranges from microgram/milligram yields all the way up to multi-gram batches.

We Are Equipped To Carry Out Large & Small Scale Chromatography

- Affinity
- Size Exclusion
- Ion Exchange
- Hydrophobic Interaction

Reverse phase chromatography under controlled temperature conditions

We are undertaking protein and peptide purification process development projects, offering customers a way to take advantage of our extensive background in the scale up process.

Our Laboratories Are Equipped With:

- Multiple columns ranging from 1mL to 7L
- Purification systems with flow rates ranging 1mL /min to 1L/min for low, medium and high pressure chromatography
- MALDI and ESI mass spectrometer
- Multiple Analytical HPLC systems for quality control analysis
- Concentration of samples by Roto-Vap
- Gel imaging systems and densitometry for various types of protein and peptide detection
- Programmable shelf lyophilizers for final formulation under controlled cooling and drying conditions
- Dynamic axial compression columns for up to 1L bed volume, with packing station, which gives us the capability of custom packing for scaling up high and medium pressure separations
- Contractual partners that can carry out 5L to 1000L fermentations.
- Gel images and Plate readers for performing ELISA, protein concentration assay, testing for endotoxin, and many other quality control analysis procedures.
Contract Services

For quality monitoring we have the capability to conduct analytical reverse phase and size exclusion chromatography, SDS-polyacrylamide gel electrophoresis, western blots, ELISA, protein quantification assays, and mass spectrometry analysis. We are also equipped with dual stage shelf lyophilizers for drying of protein in an organic or aqueous environment, and have extensive experience in developing lyophilization protocols.

Our Process Development & Optimization Services Include

- Expression and purification of Non-Radioactive Labeled Peptides and Proteins
- Development of lyophilization protocols for final formulations
- Column matrix screening for multiple chemistries
- Protease cleavage condition optimization
- Inclusion body solubilization and refolding
- Quality Control Assay development

Custom Protein Purification Projects Are Typically Carried Out In 3 Phases

1. Method development which includes 1mL-5mL column screening. Multiple steps may have to be optimized depending on specifications of the final purity required by client. QC analysis of purity and check of bioactivity as per client requirement. In addition, final formulation screening can be performed during this phase.

2. Transfer of the method developed in phase 1 to a 200mL-500mL level for scalability assessment.

3. A 1L-7L prep scale run is completed based on the processes developed in phases one and two.

All materials, data, and records generated through each phase will be delivered to the client for approval before proceeding to the next phase. All projects and details will be treated with utmost confidentiality and rights to the protocol developed and the material are the property of the client. The pricing will be quoted for each phase. A project can be terminated after a completed phase if the results do not meet the clients’ expectation. Clients are welcome to use rPeptide’s service for one or all three of these phases. rPeptide strives to provide quality services that meet all customer’s needs with an emphasis on producing consistent results on budget and on time.