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Anti-TAU-1 Monoclonal Antibody

CLT9007 Lot: LV1383492

DESCRIPTION: Anti-TAU-1 microtubule-associated protein

PRESENTATION: 100 µg, liquid (1 mg/ml concentration). Presented in 10 mM potassium phosphate, 70 mM NaCl, pH 7.4.

DESCRIPTION:

Biological role:

Tau is one of several high molecular weight proteins that play an important role in brain microtubule assembly (1,2). In vitro, tau co-purifies with brain microtubules, maintains a constant stoichiometry to tubulin through several cycles of temperature-dependent assembly and disassembly, and lowers the critical concentration for microtubule assembly.

Tau may play a role in Alzheimer's disease. Antibodies specific for different isoforms of tau, and antibodies specific for an epitope common to all tau isoforms, stain neurofibrillary tangles in post-mortem brain tissues from individuals with a confirmed diagnosis of Alzheimer's disease (3). Cedarlane's anti-tau monoclonal antibody stains neurofibrillary tangles in phosphatase-treated tissue sections (6-8). Cellular and subcellular localization:

<u>In situ</u>, anti-tau has a stringent specificity for the axons of neurons. The antibody does not stain the cell bodies or dendrites of neurons, nor does it stain any other cell type (4). However, this *in vivo* intracellular specificity is not maintained in culture: anti-tau stains the axons, cell bodies and dendrites of rat hippocampal neurons grown in culture(5).

The specificity of anti-tau was originally thought to represent the restricted expression of tau to axons. Later studies revealed that this specificty is dependent on the state of phosphorylation. In dephosphorylated samples (samples treated with alkaline phophatase), anti-tau stains astrocytes, perineuronal glial cells, and the axons, cell bodies and dendrites of neurons,

while in untreated samples, anti-tau stains only axons (6). (The epitope recognized by anti-tau is probably at or near a phosphorylated site.)

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Electrophoretic profile:

The electrophoretic profile of tau is heterogeneous. In brain microtubules purified by two cycles of assembly and disassembly, tau is observed as a single electrophoretic species in guinea pig, as three species in mouse, and four species in cow (1). Four to five electrophoretic species are present in the adult rat brain (2), having molecular weights of 5 2-68 kDa. In human brain, four to six electrophoretic species of tau are present, having molecular weights of 50-68 kDa (one dimensional SDS page).

SPECIFICITY:

Anti-tau binds to all known electrophoretic species of tau in human, rat and bovine brain (one-dimensional SDS-PAGE).

IMMUNOGEN: Purified denatured bovine microtubule associated proteins.

<u>CLONE</u>: Tau-1 (PC1C6) (4)

<u>SUBTYPE</u>: Mouse IgG2a (4)

PREPARATION:

BALB/c mice were immunized with purified, denatured bovine microtubule associated proteins. Lymphocytes from the immunized mouse spleens were fused with mouse Sp2/0 myeloma cells (4). Antibody was harvested from cells cultured in fetal bovine serum-supplemented medium and purified by ammonium sulfate precipitation and anion exchange chromatography.

STORAGE/STABILITY:

Maintain frozen at -20°C in undiluted aliquots for up to 24 months. Avoid repeated freeze/thaw cycles.

ANALYSIS:

Purity ≥90% as determined by SDS-PAGE with Coomassie blue staining or HLPC.

WESTERN BLOT ANALYSIS:

Bovine brain microtubule proteins purified by two cycles of assembly and disassembly (9) are dissolved in SDS-PAGE sample buffer. Five micrograms of the microtubule preparation per lane is loaded onto a 4% to 20% SDS-PAGE gradient gel alongside molecular weight markers (14.3-200 kDa). After separation by electrophoresis, the proteins are blotted onto nitrocellulose. Tau is detected as a series of 5 bands (52-68 kDa) with approximately 5 ng/ml anti-tau-1.

APPLICATIONS:

Immunohistochemistry:

Anti-tau can be used to stain tissue (brain or spinal cord) fixed with paraformaldehyde. Below is a procedure developed to examine tau distribution in coronal sections of rat hippocampus.

Working Concentration: approximately 5 µg/ml.

Preparation of slides:

- 1. Perfuse rat brain with 4% paraformaldehyde.
- 2. Cut 10-20 µm cryostat sections of the brain or brain structure of interest.
- 3. If using a peroxidase secondary antibody, block endogenous peroxidase activity by incubating tissue sections in phosphate buffered saline (PBS) containing 0.03% H_20_2 for 15 min. at room temperature.
- 4. Rinse 3 times with PBS, 3 min. per rinse.

Dephosphorylation of tissue sections (optional)

Dephosphorylation with alkaline phosphatase is recommended for staining neurofibrillary tangles in Alzheimer's brain tissue with anti-tau (6). This treatment change the staining pattern of anti-tau to include the cell bodies, dendrites and axons of neurons. In untreated samples, anti-tau stains axons only.

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 Incubate tissue sections at 32°C for 2.5 hours with constant agitation in the following solution: 100 mM Tris HCI, pH 8.0 130 units/ml alkaline phosphatase 1 mM PMSF

- 10 µg/ml pepstatin
- 10 µg/ml leupeptin
- 2. Rinse sections twice, 3 min per rinse, with 100 mM Tris HCI, pH 8.0.
- 3. Proceed with anti-tau staining procedure (below).

Anti-Tau Staining:

- 1. To block non-specific binding, incubate sections in PBS containing 1% (v/v) normal animal serum and 0.03% (w/v) Triton®-X 100. The serum source should be from the same species as the secondary antibody.
- 2. Rinse 3 times with PBS, 3 min. per rinse.
- 3. Incubate sections in anti-tau-1 (~5 µg/ml) diluted in PBS containing 1% normal animal serum.
- 4. Wash with PBS, change solution 3 times over a 3 minute period.
- 5. Detect with a standard secondary antibody detection system (10-13).

Western Blot Analysis:

Anti-tau can be used to identify tau species in Western blots. See description above (under Analysis) for conditions.

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