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Catalogue# Prot-m-NF-H-Por: Purified Porcine Neurofilament Heavy Chain

Background: Neurofilaments are the 10 nm or intermediate filament proteins found specifically in neurons, and are composed predominantly of four major proteins called NF-L, NF-M, NF-H and α-internexin (1). NF-H is the neurofilament heavy or high molecular weight polypeptide and runs on SDS-PAGE gels at 200-220kDa in the heavily phosphorylated axonal form. The molecule has an unusual and interesting region consisting of multiple Lysine-Serine-Proline peptides, about 40 of these in human. These peptide repeats are heavily phosphorylated on the Serine residues in axons. Enzymatic removal of these phosphate groups will increase the SDS-PAGE mobility to about 160kDa, likely due to protein conformational changes due to the removal of charge (2). Even the non-phosphorylated form runs aberrantly on SDS-PAGE, as the real molecular weight of NF-H is about 110kDa, with some variation in different species. This is likely due to an unusually high content of charged amino acids. Non-phosphorylated forms of NF-H are found in dendrites and perikarya and early in development, but the majority of NF-H in the adult is this heavily phosphorylated axonal form. Our preparation was isolated from pig spinal cord using a modification of the method of Leung and Liem (3), which purifies out the heavily phosphorylated axonal form, often referred to as pNF-H. The HGNC name for this protein is NEFH.

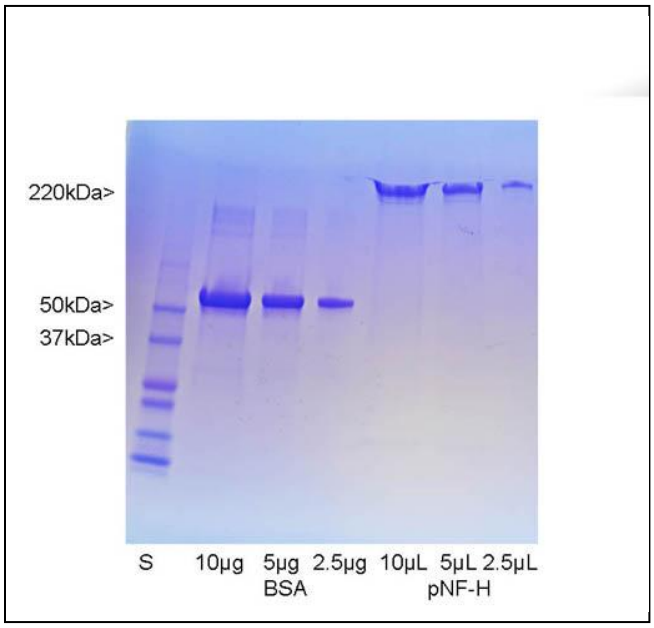

Figure: Coomassie brilliant blue stained SDS-PAGE gel of pig pNF-H preparation. First lane shows molecular weight standards of indicated molecular size. Next three lanes show BSA at 10, 5 and 2.5µg respectively. Next three show the indicated volumes of the pNF-H preparation. The pNF-H was isolated from pig spinal cord using the Triton X100/sucrose extraction method of Leung and Liem as far as the 6M urea solubilization and batch ion exchange chromatography on hydroxyapatite steps (3). The material was then further fractionated using a phosphate gradient of 10mM to 300mM on DEAE-cellulose in 6M urea at pH=7.5. The pNF-H elutes cleanly at about 50mM phosphate as shown. The pig pNF-H runs on SDS-PAGE with an apparent molecular weight of 220kDa.

Protein Characteristics: Purified protein is concentrated at 1.0 mg/mL and is supplied in 6M urea, 10mM phosphate buffer at pH=7.5.

References:

1. Perrot, R., Berges, R., Bocquet, A and Eyer, J. Review of the multiple aspects of neurofilament functions, and their possible contribution to neurodegeneration. Mol. Neurobiol 38:27-65 (2008).
2. Julien, J-P. and Mushynski, W. E. Multiple phosphorylation sites in mammalian neurofilament polypeptides. J. Biol. Chem. 257:10467-10470 (1982).
3. Leung, C. L. and Liem, R. K. H. Isolation of intermediate filaments. Curr. Prot. Cell Biol. 3:Unit 3.23 doi: 10.1002/0471143030.cb0323s31 (2006).

Storage: Store at -20°C or lower temperatures. Avoid repeat freezing and thawing. Keep container closed and protected from light and moisture. Valid for one year from ship date.

Limitations: This product is for research use only and is not approved for use in humans or in clinical diagnosis.