Monoclonal Mouse Anti-Human Neurofilament Protein
Clone 2F11
Code No. M 0762
Lot 071. Edition 04.03.03

Intended use
For in vitro diagnostic use.
Monoclonal Mouse Anti-Human Neurofilament Protein, Clone 2F11, is intended for use in immuno-cytochemistry. The antibody labels neurons (axons) of the central and peripheral nervous system (1, 2), and is a useful tool for the identification of tumours with neuronal differentiation (1, 3). The antibody can also be used to discriminate between Hirschsprung’s disease and allied enteric nervous system malformations (2). Differential identification is aided by the results from a panel of antibodies – especially antibodies against other types of intermediate filaments. Interpretation must be made within the context of the patient’s clinical history and other diagnostic tests by a qualified pathologist.

Introduction
Neurofilaments (NFs) belong to the family of intermediate filaments (IFs) and are structural elements of the neuronal cytoskeleton in an interconnection with actin microfilaments, microtubules and other IFs. NFs are composed of three different subunits that are different, but related proteins: NF-L (70 kDa), NF-M (150-160 kDa) and NF-H (200 kDa). The antigenic determinants of each of the subunits may be unique or shared and each NF subunit is a separate gene product. During embryonic neurogenesis, the NF-L and NF-M subunits are coexpressed, whereas the activation of the NF-H subunit is delayed to the postnatal period. NF-M and NF-H subunits are unable to selfassemble and, typically, form co-polymers with NF-L (4, 5).

Reagent provided
Monoclonal mouse antibody provided in liquid form as cell culture supernatant dialysed against 0.05 mol/L Tris/HCL, pH 7.2, and containing 15 mmol/L NaN₃.
Clone: 2F11 (6). Isotype: IgG1, kappa.
Mouse IgG concentration: 270 mg/L. Total protein concentration: 16.3 g/L.

Immunogen
Neurofilament isolated from normal adult human brain (6).

Specificity
In immunoblotting, the antibody reacts with the 70 kDa subunit of neurofilament (6). In immunocytochemistry, the antibody specifically labels neuronal cells (6).
As demonstrated by immunocytochemistry, the antibody cross-reacts with the NF-equivalent protein in opossum (7), cat, cow, dog, horse, mouse, rabbit, rat and swine.

Precautions
1. For in vitro diagnostic use.
2. This product contains sodium azide (NaN₃), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.

Storage
Store at 2-8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the user must verify the conditions. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact our Technical Services.

Specimen preparation
Paraffin sections: The antibody can be used for labelling paraffin-embedded tissue sections fixed in formalin. Pretreatment of tissues with heat-induced epitope retrieval is recommended. Optimal results are obtained with DakoCytomation Target Retrieval Solution, code No. S 1700, DakoCytomation Target Retrieval Solution, High pH, code No. S 3308, 10 mmol/L citrate buffer, pH 6.0, or 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0. Pretreatment of tissues with proteinase K was found less efficient. The tissue sections should not dry out during the treatment of during the following immunocytochemical staining procedure.
Frozen sections and cell preparations: The antibody can be used for labelling frozen sections (3, 7).

Staining procedure
Dilution: Monoclonal Mouse Anti-Human Neurofilament Protein, code No. M 0762, may be used at a dilution range of 1:50-1:100 when applied on formalin-fixed, paraffin-embedded sections of human colon and using 20 minutes heat-induced epitope retrieval in DakoCytomation Target Retrieval solution, code No. S 1700, and 30 minutes incubation at room temperature with the primary antibody. Optimal conditions may vary depending on specimen and preparation method, and should be determined by each individual laboratory. The recommended negative control is DakoCytomation Mouse IgG1, code No. X 0931, diluted to the same mouse IgG concentration as the primary antibody.
Visualization: DAKO LSAB™+/HRP kit, code No. K 0679, and DAKO EnVision™+/HRP kits, code Nos. K 4004 and K 4006, are recommended. For frozen sections and cell preparations, the DakoCytomation APAAP kit, code No. K 0670, is a good alternative if endogenous peroxidase staining is of concern. Follow the procedure enclosed with the selected visualization kit.

Automation: The antibody is well suited for immunocytochemical staining using automated platforms, such as the DakoCytomation Autostainer.

Performance characteristics

Cells labelled by the antibody display a cytoplasmic staining pattern.

Normal tissues: In sections of normal colon, the antibody labels some axons in the axon bundles of the plexus of Auerbach and Meissner (2, 6), whereas the perikarya of the ganglion cells are not immunostained (6).

Abnormal tissues: In cases of Hirschsprung’s disease, the antibody strongly labels axons in the plexus of Auerbach and Meissner in the aganglionic bowel segments (2, 6). In gangliogliomas, the antibody labelled neuronal processes in 10 of 13 tumours, whereas only in 5 of the cases, significant staining was observed in the neuronal perikarya (1). In an immunocytochemical study of intermediate filaments in Merkel cell tumours, 2 of 2 frozen sections were positive in virtually all tumour cells in a diffuse reticular and focal granular pattern. In formalin-fixed, paraffin-embedded tissue, 2 of 8 cases were positive (3). In frozen sections of 94 lung tumours, coexpression of cytokeratins and NF was observed in 22%. The labelling was primarily focal or patchy (8).

References


M 0762/HEW/04.03.03