Monoclonal Mouse Anti-Human Cytokeratin
Clone MNF116
Code No. M 0821
Lot 061. Edition 08.08.01

Intended use
For in vitro diagnostic use.
DAKO Monoclonal Mouse Anti-Human Cytokeratin, Clone MNF116, is intended for use in immunocytochemistry. The antibody labels epithelial tissues from simple glandular to stratified squamous epithelium, and is a useful tool for the identification of normal and neoplastic cells of epithelial origin (1, 2). Differential identification is aided by the results from a panel of antibodies. Interpretation must be made within the context of the patient’s clinical history and other diagnostic tests by a qualified pathologist.

Introduction
The cytokeratins (CKs) belong to the intermediate filaments, which create a cytoskeleton in almost all eukaryotic cells. In contrast to other intermediate filaments, CKs are made up of a highly complex multigene family of polypeptides with molecular masses ranging from 40 to 68 kDa. CKs are generally held to belong to the most fundamental markers of epithelial differentiation, and until now, 20 distinct CK polypeptides have been revealed in various human epithelia (3). The CKs can be divided into an acidic type A (class I) and a neutral-basic type B (class II) subfamily.

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. Package size is 1mL.
Mouse IgG concentration: 190 mg/L. Total protein concentration: 5.8 g/L.

Immunogen
Crude extract of splenic cells from a nude mouse engrafted with MCF-7 cells (human breast carcinoma cell line).

Specificity
The antibody is a broad spectrum anti-keratin reagent reacting with intermediate and low-molecular-weight keratins. Thus in immunoblotting, the antibody labels a number of discrete bands ranging from 40 to 58 kDa, corresponding to cytokeratins 5, 6, 8, 17 and probably 19.
As demonstrated by immunocytochemistry, the antibody cross-reacts with cytokeratin in various mammals, including cow (4), mouse (5) and rabbit (6).

Precautions
1. For in vitro diagnostic use.
2. The NaN₃ used as a preservative is toxic if ingested. NaN₃ may react with lead and copper plumbing to form highly explosive metal compounds. Upon disposal, flush with large volumes of water to prevent 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 DAKO Technical Services.

Specimen preparation
Paraffin sections: The antibody can be used for labelling paraffin-embedded tissue sections fixed in formalin. Optimal immunostaining is obtained when using proteinase K for epitope retrieval. However, trypsin (1, 7) and pepsin (2) have also been applied successfully. Additionally, satisfactory staining is seen after heat-induced epitope retrieval in 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0. The tissue sections should not dry out during the treatment or during the following immunocytochemical staining procedure.
Frozen sections and cell preparations: The antibody can be used on frozen sections as demonstrated with the DAKO EPOS-Conjugate, Clone MNF116, code No. U 7022. (8).

Staining procedure
Dilution: DAKO Monoclonal Mouse Anti-Human Cytokeratin, code No. M 0821, may be used at a dilution range of 1:50-1:100 when applied on formalin-fixed, paraffin-embedded sections of human tonsil and using 10 minutes proteinase K treatment of tissues, 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. As negative control, DAKO Mouse IgG1, code No. X 0931, diluted to the same mouse IgG concentration as the primary antibody, is recommended.
Visualization: DAKO LSAB®+/HRP kits, code Nos. K 0679 and K 0690, and DAKO EnVision™+/HRP kits, code Nos. K 4006 and K 4007, are recommended. For frozen sections and cell preparations, DAKO APAAP kit, code No. K 0670, is a good alternative if endogenous peroxidase staining is a 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 DAKO Autostainer.

Performance characteristics

Cells labelled by the antibody display a cytoplasmic staining pattern.

Normal tissues: The antibody labels a number of different epithelia, e.g. simple epithelia in biliary and pancreatic ducts, proximal and distal convoluted renal tubules, thyroid-, parathyroid-, gastric-, gall bladder-, small intestinal-, large intestinal-, and endometrial epithelium, as well as hepatocytes (1). The epithelial lining of the rete ovarii, epoophoron, Fallopian tube and ovarian surface is also reactive with the antibody (7). In stratified squamous epithelium of skin, the antibody labels the basal layer, as well as the outer root sheath of follicular epithelium, and the basal layer of sebaceous, eccrine, and apocrine glands and ducts (2). Non-squamified stratified epithelia of esophagus and ectocervix are also reported positive with the MNF116 antibody (1). Transitional epithelium (urothelium) and different complex epithelia, e.g. ducts and acini of breast, endocervical glands, prostate epithelium, epididymis, bronchial epithelium as well as ducts and acini of salivary glands are also labelled by the antibody (1). Mesenchymal cells are generally not reactive with the antibody, however a weak and focal labelling of smooth muscle cells is reported. Endothelium, skeletal muscle, cartilage, and lymphoid tissues are not labelled by the antibody (1).

Abnormal tissues: Of epithelial tumours, the antibody labelled 4/4 colorectal carcinomas, 3/3 gastric carcinomas, 4/4 breast carcinomas, 3/3 prostatic carcinomas, 3/3 renal cell carcinomas, 3/4 hepatocellular carcinomas, 3/3 transitional cell carcinomas, 2/3 carcinoids of appendix, and, moreover, 2/2 teratomas and 2/2 pleomorphic adenomas (epithelial elements), and 14/14 squamous cell carcinomas, i.e. 6 of epidermal, 3 of cervical- and 5 of bronchial origin (1). In addition, 16/16 cases of mixed tumours and myoepitheliomas rising in soft tissues, displayed positive reactivity with the antibody (9). Positive labelling of mesothelioma has also been observed. The antibody, further, labels micrometastatic cells of non-small cell lung carcinomas in lymph nodes (10). Non-epithelial tumours, e.g. melanomas, lymphomas, benign and malignant fibrous histiocytomas, leiomymomas, liposarcomas, chondrosarcomas, Ewing’s sarcomas, angioloblastomas, angiosarcomas, atypical fibroxanthomas, juvenile xanthogranulomas, hemangiomas, granular cell tumours, hair follicle myxomas, epithelial histiocytomas and dermal fibrosarcomas are not labelled by the antibody. However, in 1/6 cases (1), and 1/2 cases (2) of leiomyosarcomas, the antibody displayed a weak and focal reactivity.

References