Monoclonal Mouse Anti-Human Epithelial Antigen
Clone Ber-EP4
Code No. M 0804
Lot 062. Edition 19.07.02

Intended use
For in vitro diagnostic use.
DAKO Monoclonal Mouse Anti-Human Epithelial Antigen, Clone Ber-EP4, is intended for use in immunocytochemistry. The antibody labels most epithelial cells and is useful as a discriminant in the differential diagnosis of adenocarcinoma versus malignant mesothelioma (1). The antibody may also aid in the detection of micrometastases in lymph nodes of patients with oesophageal carcinoma (2) and in differentiating between basal and squamous cell carcinomas of the skin (3). 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
Epithelial antigen is a cell surface glycoprotein of unknown function (4). This epithelium-specific antigen is broadly distributed in epithelial cells, and displays a highly conserved expression in carcinomas (4, 5). As exceptions to the general expression in normal epithelia, adult hepatocytes, in contrast to foetal hepatocytes, parietal cells in gastric glands, and apical cells in squamous epithelia are negative. Epithelial antigen is rarely present in mesotheliomas (1, 4).

It has been reported that epithelial antigen plays an important role as tumour-cell marker in lymph nodes from patients with oesophageal carcinoma otherwise classified as node-negative (2). Epithelial antigen has also been suggested as a discriminator between basal cell and basosquamous carcinomas, and squamous cell carcinoma of the skin (3).

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.
Mouse IgG concentration: 320 mg/L.

Immunogen
MCF-7 cells (human breast carcinoma cell line) (4).

Specificity
Analysis of immunocomplexes between the antibody and lysate of 125I surface-labelled MCF-7 cells in SDS-PAGE under reducing conditions shows that the antibody labels two polypeptides of 34 kDa and 39 kDa, respectively, corresponding to epithelial antigen. Under non-reducing conditions, the polypeptides appear as 39 kDa and 41 kDa, while deglycosylation reduces the size to 31 kDa, and 36 kDa.

In immunoprecipitation experiments, the Ber-EP4 antibody blocks the reaction of the HEA125 antibody with MCF-7 cell lysate and vice versa, showing that the two antibodies react with the same antigen. The two antibodies also produce identical staining results in cells and tissues (4).

Of 37 cell lines tested, the antibody homogeneously labels all (10/10) carcinoma cell lines, whereas all non-epithelial cell lines (26/27) are negative except for the erythromyeloid cell line K562 (4).

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 DAKO Technical Services.

Specimen preparation
Paraffin sections: The antibody can be used for labelling paraffin-embedded tissue sections fixed in formalin.
Pre-treatment of tissues with heat-induced epitope retrieval is required. For heat-induced epitope retrieval, DAKO Target Retrieval Solution, code No. S 1700, is recommended. 10 mmol/L citrate buffer pH 6 is less efficient, and 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0, and DAKO Target Retrieval Solution, High pH, code No. S 3308, are inefficient. Pre-treatment of tissues with proteinase K gives non-optimal labelling. 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 for labelling acetone-fixed, frozen sections and cell preparations (4).

**Staining procedure**

**Dilution:** DAKO Monoclonal Mouse Anti-Human Epithelial Antigen, code No. M 0804, may be used at a dilution range of 1:200-1:400 when applied on formalin-fixed, paraffin-embedded sections of human kidney and using 20 minutes heat-induced epitope retrieval in DAKO 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 DAKO 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 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.

**Product-specific limitations**

In formalin-fixed, paraffin-embedded carcinomas arising from tissues with no or smaller amounts of epithelial antigen, such as hepatocellular and lung carcinomas, the antibody does not perform satisfactorily compared to labelling in frozen sections of the same tissues (4). Owing to the relative lability of the epitope in formalin-fixed, paraffin-embedded tissue sections, negative results should be interpreted with caution (4).

**Performance characteristics**

Cells labelled by the antibody display membrane and cytoplasmic staining. The membrane staining is preferentially basolateral (4).

**Normal tissues:** All normal epithelial tissues are labelled by the antibody. Epithelial cells of different origin display varying levels of staining, but most epithelia are strongly positive. Only paticelial cells in gastric glands, apical cell layers in squamous epithelia, and adult hepatocytes are negative (4). The antibody does not label non-epithelial tissues, including spleen, peripheral blood, bone marrow, brain, connective tissue, smooth and striated muscle, heart, endothelia, and myoepithelia. Additionally pleura and peritoneum-lining cells are negative, but cells covering the ovary display a slight staining (4).

**Abnormal tissues:** The antibody labelled 142 of 144 epithelial tumour specimens, irrespective of their differentiation, derived from breast, oesophagus, stomach, colon, rectum, pancreas, kidney, liver, lung, thyroid and salivary glands, vagina, ovary, cervix uteri and nasopharynx, reflecting the staining pattern in their non-malignant counterparts. Hepatocellular carcinomas displayed heterogeneous staining and included the two negative cases. In this study (4), 2 of 2 squamous cell carcinomas of the lung and cervix uteri, respectively, were positive in formalin-fixed, paraffin-embedded specimens, even though only the basal cell layers were labelled in normal tissues. In some carcinomas, such as gastric carcinomas, the antibody demonstrated a stronger labelling than in normal tissues, especially on the membrane. None of 88 non-epithelial tumours and 20 cases of leukaemia were labelled by the antibody (4).

In a study of 83 adenocarcinomas and 115 malignant mesotheliomas, 72/83 (87%) adenocarcinomas were labelled by the antibody whereas only 1/115 (0.9%) malignant mesotheliomas was labelled (1). In another study, 20/20 (100%) lung adenocarcinomas and 4/46 (9%) mesotheliomas were labelled. Of the 4 positive mesotheliomas, the 2 showed a strictly focal labelling (6).

In lymph nodes classified as tumour free by conventional histopathological staging, the antibody labelled micrometastatic tumour cells in 89 of 126 patients (71%) with completely resected oesophageal carcinomas (2).

In a study of 75 skin tumours, the antibody labelled 39/39 basal cell carcinomas, 0/23 squamous cell carcinomas, and showed some areas of staining in 13/13 basosquamous carcinomas, and showed some areas of staining in 13/13 basosquamous carcinomas (3).

**References**