**Monoclonal Mouse Anti-Vimentin**

**Clone V9**

**Code No. M 0725**

**Lot 092. Edition 16.09.02**

### Intended use

For in vitro diagnostic use.

DAKO Monoclonal Mouse Anti-Vimentin, Clone V9, is for use in immunocytochemistry. The antibody labels primarily cells of mesenchymal origin in normal and neoplastic tissues, and is of value in tumour diagnosis. Differential identification is aided by the results from a panel of antibodies – especially antibodies against other types of intermediate filaments (1). Interpretation must be made within the context of the patient’s clinical history and other diagnostic tests by a qualified pathologist.

### Introduction

Vimentin is a 57 kDa intermediate filament (IF) protein, which form part of the cytoskeleton of vertebrate cells. Among the five classes* of IFs, comprising nine groups, vimentin belongs to class III, showing a high degree of specificity for cells of mesenchymal origin. The cell type specificity, displayed by each of the IF subtypes, was initially thought to be retained in malignant cells as well as their normal counterpart, which made IF’s important as diagnostic markers in histogenesis. The coexpression of intermediate filaments, particularly vimentin and cytokeratin, has now been demonstrated in a variety of normal cells/tissues and in neoplastic lesions, necessitating the use of a panel of antibodies in differential tumour diagnosis (2).

*Recently, an additional class (i.e. class VI) was created for nestin (2).

### 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:** V9 (3). **Isotype:** IgG1, kappa.

**Mouse IgG concentration:** 360 mg/L. **Total protein concentration:** 19.3 g/L.

### Specificity

In Western blotting of purified porcine vimentin, the antibody labels a single band of 57 kDa corresponding to vimentin. When applying whole cell extracts of cell lines expressing vimentin plus glial fibrillary acidic protein (GFAP), and vimentin plus desmin, respectively, the antibody labels specifically the 57 kDa vimentin band. As directly shown by these experiments, the antibody does not react with the two IF proteins most closely related to vimentin, i.e. desmin and GFAP (3).

In immunocytochemistry the antibody labels the vimentin-positive human cell lines IMR90, RD, glioma and HeLa (3).

As demonstrated by immunocytochemistry, the antibody cross-reacts with the vimentin-equivalent protein in man, cow, dog, hamster, horse, rhesus and African green monkey, rabbit, rat and rat kangaroo. No cross-reaction with mouse vimentin could be demonstrated, and results on chicken specimens are contradictory (3, 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. Heat-induced epitope retrieval in 10 mmol/L citrate buffer, pH 6.0, or in DAKO Target Retrieval Solution, code No. S 1700, is recommended. The tissue sections should not dry out during the treatment or during the following immunocytochemical staining procedure.

**Frozen sections or cell smears:** The antibody can be used for labelling frozen sections or fixed cell smears (3).

### Staining procedure

**Dilution:** DAKO Monoclonal Mouse Anti-Vimentin, code No. M 0725, 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 15 minutes heat-induced epitope retrieval in 10 mmol/L citrate buffer, pH 6.0, 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.

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(2)
Visualization: DAKO LSAB®+/HRP kit, code No. K 0679, and DAKO EnVision™+/HRP kits, code Nos. K 4004 and K 4006, are recommended. The APAAP or LSAB techniques are recommended for acetone-fixed frozen sections. 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: In general, most human mesenchymal cells are labelled by the antibody, including fibrocytes, lipocytes, smooth muscle cells, vascular endothelial cells, astrocytes, peripheral nerve (Schwann) cell, macrophages (including Kupfer cells), as well as myoepithelial cells of sweat and salivary glands and of breast, which are all labelled strongly. Also positive, with variable intensity and distribution, are the follicular cells of the thyroid, adrenal cortex, renal distal tubules, and mesangial and endothelial cells of the renal glomerulus, as well as pancreatic acinar cells (1, 3). In the human eye, the antibody labels the pigmented posterior and the anterior epithelia of the human iris, including the muscle portion (dilator pupillae) of the anterior epithelium, as well as the nonpigmented and pigmented ciliary epithelia (5). In the ciliary epithelia, vimentin was coexpressed with cytokeratin (5). Skeletal and cardiac muscle cells, epidermal, squamous, urothelial, colonic and gastric mucosal, and glial cells, as well as neurons are consistently negative with the antibody (1, 3).

Abnormal tissues: The antibody labelled 17/20 sarcomas, 16/18 melanomas, 4/4 meningeomas, and 3/3 Schwannomas, and was the sole intermediate filament present in these tumours. In addition, variable percentages (10-57%) of carcinomas, neuroendocrine carcinomas, neuroblastomas, thymomas and mesotheliomas were positive with the antibody. With the exception of the neuroblastomas, cytokeratin was coexpressed with vimentin in these tumours. Among adenocarcinomas, more than 50% of papillary carcinomas of the thyroid as well as renal, endometrial, ovarian and lung carcinomas were labelled by the antibody and coexpressed keratins and vimentin (1).

References


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