Monoclonal Mouse Anti-Human Smooth Muscle Actin
Clone 1A4
Code No. M 0851
Lot 032, Edition 14.03.02

Intended use
For in vitro diagnostic use.
DAKO Monoclonal Mouse Anti-Human Smooth Muscle Actin, Clone 1A4, is intended for use in
immunocytochemistry. The antibody labels smooth muscle cells, myofibroblasts and myoepithelial cells, and it
is a useful tool for the identification of leiomyomas, leiomyosarcomas (1, 2), and pleomorphic adenomas (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
Cytoplasmic actins, which belong to the microfilament system of cytoskeleton proteins, are some of the most
conserved eukaryotic proteins being expressed in mammals and birds. The actin protein consists of six
isoforms, varying in their amino acid sequence, but all having the same molecular mass of 42 kDa. The
isoforms show more than 90% overall sequence homology, but only 50-60% homology in their 18 N-terminal
residues. The N-terminal region appears to be a major antigenic region (4). There are different α isoforms
specific for muscle tissues, i.e. skeletal muscle α, cardiac muscle α, and smooth muscle α, respectively (1).
The β- and γ-actins may be present in muscle cells as well as most other cell types in the body, including non-
muscle cells (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: 1A4*. Isotype: IgG2a, kappa.
(* The antibody from this clone is identical to the anti-asm-1 described in (4)).
Mouse IgG concentration: 70 mg/L. Total protein concentration: 4.2 g/L.

Immunogen
N-terminal synthetic decapeptide of α-smooth muscle actin coupled to keyhole limpet haemocyanin (KLH) (4).

Specificity
The antibody reacts specifically with the α-smooth muscle isoform of actin as determined by Western blotting
and 2D-PAGE immunoblotting (4).
As demonstrated by Western blotting and/or immunocytochemistry, the antibody cross-reacts with the α-
smooth muscle actin-equivalent protein in chicken, cow and rat (4).

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. Pre-
treatment of tissues with heat-induced epitope retrieval is recommended. For heat-induced epitope retrieval, the
following solutions were found efficient: 10 mmol/L citrate buffer, pH 6.0; 10 mmol/L Tris buffer, 1 mmol/L EDTA,
pH 9.0; DAKO Target Retrieval Solution, code No. S 1700; or DAKO Target Retrieval Solution, High pH, code No.
S 3308. Pre-treatment of tissues with proteinase K was found inefficient for epitope retrieval. 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 acetone-fixed, frozen sections (1).

Staining procedure
Dilution: DAKO Monoclonal Mouse Anti-Human Smooth Muscle Actin, code No. M 0851, may be used at a
dilution range of 1:50-1:100 when applied on formalin-fixed, paraffin-embedded sections of normal human
colon, 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. As negative control, DAKO
Mouse IgG2a, code No. X 0943, diluted to the same mouse IgG concentration as the primary antibody, is
recommended.
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, 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.

(2)
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 smooth muscle cells in blood vessels and, additionally, salivary ducts and myoepithelial cells around acini in salivary glands (3). Smooth muscle cells in 35/36 normal uterine myometria were also positively labelled (2). Further, a temporal labelling of perisinusoidal liver cells has been observed (6). In frozen tissues, the antibody labels myofibroblasts and myoepithelial cells around acini and ducts of the breast, whereas epithelia (adeno, squamous), lymphocytes, cardiac- and skeletal muscle cells, endothelial cells, fat cells, Schwann cells and fibroblast are negative (1).

Abnormal tissues: The antibody labelled 24/26 leiomyomas, 6/7 atypical leiomyomas and 21/25 leiomyosarcomas of the uterus, as well as 13/13 extraterine nongastrointestinal spindled leiomyosarcomas (2). Moreover, the antibody labelled a variable amount of cells in 8/8 pseudosarcomatous myofibrolastic tumours of the urinary bladder in children (7). In pleomorphic adenomas, the antibody labelled tumour epithelial cells (myoepithelial cells) in 19/20 cases (3). In frozen tissues, the antibody, in addition to the labelling of 5/5 leiomyomas and 6/7 leiomyosarcomas, also labelled 4/22 malignant fibrous histiocytomas and 1/2 rhabdomyosarcomas. 6/6 malignant schwannomas were negative, as also 13/13 other soft tissue tumours, including 1 fibrosarcoma, 6 liposarcomas, 1 angio-sarcoma, 1 capillary haemangioma, 1 Triton tumour, and 3 synovial sarcomas (1).

References