DATA SHEET

Order No. IS70030
30 tests, 6 mL

FLEX
Monoclonal Mouse
Anti-Human
Muscle Actin
Clone HHF35
Ready-to-Use
(Dako Autostainer/Autostainer Plus)

Code IS700

ENGLISH

Intended use

For in vitro diagnostic use.

FLEX Monoclonal Mouse Anti-Human Muscle Actin, Clone HHF35, Ready-to-Use (Dako Autostainer/Autostainer Plus), is intended for use in immunohistochemistry together with Dako Autostainer/Autostainer Plus instruments. This antibody is useful for the identification of soft tissue tumors with muscle differentiation, i.e. leiomyoma (LM), leiomyosarcoma (LMS) (1), and rhabdomyosarcoma (RMS) (1, 2).The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and explanation

Actin is a highly conserved protein that exists in at least six different isoforms, which can be distinguished according to their amino acid sequences and isoelectric points (1, 3). It is one of the major structural proteins and plays a key role in muscle contraction, but has also shown to regulate signal transduction, enzyme and membrane channel activity, participate in transcription, mRNA transport and translation and synaptic transmission (3).

Actin is ubiquitously expressed in a variety of cells including skeletal muscle cells, smooth muscle cells, pericytes, myoepithelial cells (4).

Refer to Dako's *General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures for: 1) Principle of Procedure, 2) Materials Required, Not Supplied, 3) Storage, 4) Specimen Preparation, 5) Staining Procedure, 6) Quality Control, 7) Troubleshooting, 8) Interpretation of Staining, 9) General Limitations.

Reagent provided

Ready-to-use monoclonal mouse antibody provided in liquid form in a buffer containing stabilizing protein and 0.015 mol/L sodium azide.

Clone: HHF35 (1, 4). Isotype: IgG1, kappa.

Immunogen

SDS extracted protein fraction of human myocardium from a case of idiopathic hypertrophic subaortic stenosis.

Specificity

In Western blotting of purified rabbit skeletal muscle actin, extracts of monkey aorta, uterus, diaphragm and heart, the antibody labels 42 kDa protein corresponding to muscle actin of α- and γ-actin isotypes, but fails to react with the α-actin of non-muscle (endothelial cells) sources (4)

Precautions

1. For professional users.

2. This product contains sodium azide (NaN_3), 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.

3. As with any product derived from biological sources, proper handling procedures should be used.

4. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.

5. Unused solution should be disposed of according to local, State and Federal regulations.

Storage

Store at 2-8 °C. Do not use after expiration date sta mped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. 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 Support.

Specimen preparation including materials required but not supplied

The antibody can be used for labeling formalin-fixed, paraffin-embedded tissue sections. Tissue specimens should be cut into sections of approximately 4 µm.

Pre-treatment with heat-induced epitope retrieval (HIER) is required using Dako PT Link (Code PT100/PT101). For details, please refer to the PT Link User Guide. Optimal results are obtained by pretreating tissues using EnVision™ FLEX Target Retrieval Solution, High pH (50x) (Code K8010/K8004).

Paraffin-embedded sections: Pre-treatment of formalin-fixed, paraffin-embedded tissue sections is recommended using the 3-in-1 specimen preparation procedure for Dako PT Link. Follow the pre-treatment procedure outlined in the package insert for EnVision™ FLEX Target Retrieval Solution, High pH (50x) (Code K8010/K8004). Note: After staining the sections must be dehydrated, cleared and mounted using permanent mounting medium.

Deparaffinized sections: Pre-treatment of deparaffinized formalin-fixed, paraffin-embedded tissue sections is recommended using Dako PT Link and following the same procedure as described for paraffin-embedded sections. After staining the slides should be mounted using aqueous or permanent mounting medium.

The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure. For greater adherence of tissue sections to glass slides, the use of FLEX IHC Microscope Slides (Code K8020) is recommended.

Staining procedure including materials required but not supplied

The recommended visualization system is EnVision™ FLEX, High pH, (Dako Autostainer/Autostainer Plus) (Code K8010). The staining steps and incubation times are pre-programmed into the software of Dako Autostainer/Autostainer Plus instruments, using the following protocols:

Template protocol: FLEXRTU2 (200 µL dispense volume) or FLEXRTU3 (300 µL dispense volume)

Autoprogram: ACTIN (without counterstaining) or ACTINH (with counterstaining)

The Auxiliary step should be set to "rinse buffer" in staining runs with ≤10 slides. For staining runs with >10 slides the Auxiliary step should be set to "none". This ascertains comparable wash times.

All incubation steps should be performed at room temperature. For details, please refer to the Operator's Manual for the dedicated instrument. If the protocols are not available on the used Dako Autostainer instrument, please contact Dako Technical Services.

Optimal conditions may vary depending on specimen and preparation methods, and should be determined by each individual laboratory. If the evaluating pathologist should desire a different staining intensity, a Dako Application Specialist/Technical Service Specialist can be contacted for information on re-programming of the protocol. Verify that the performance of the adjusted protocol is still valid by evaluating that the staining pattern is identical to the staining pattern described in "Performance characteristics".

Counterstaining in hematoxylin is recommended using EnVision™ FLEX Hematoxylin, (Dako Autostainer/Autostainer Plus) (Code K8018). Non-aqueous, permanent mounting medium is recommended.

Positive and negative controls should be run simultaneously using the same protocol as the patient specimens. The positive control tissue should include colon and tongue and the cells/structures should display reaction patterns as described for this tissue in "Performance characteristics" in all positive specimens. The recommended negative control reagent is FLEX Negative Control, Mouse, (Dako Autostainer/Autostainer Plus) (Code IS750).

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Staining interpretation

Cells labeled by the antibody display cytoplasmic staining.

Performance characteristics

Normal tissues: In colon, the smooth muscle cells in the lamina muscularis show a moderate to strong staining reaction. In tongue, the mucous/salivary glands show a weak to moderate staining reaction. In normal tissue, the antibody labels striated fibers of skeletal muscles, the smooth muscles of arteries, veins and pericytes of smaller arteries, the tunica muscularis of the gastrointestinal tract, the myometrium of the uterus, prostatic stroma, the capsule cells of several parenchymal organs, including kidney, liver, lymph nodes and spleen, and the myoepithelial layers of the mammary ducts and glands, as well as eccrine sweat, bronchial and salivary glands (1, 4, 7, 5-7). Other non-muscle cells are non-reactive, including connective tissue, epithelial cells, lymphoid cells, macrophages, neural cells, and vascular endothelial cells (1, 4, 6, 7).

Abnormal tissues: In pathological tissues, the antibody was demonstrated to be a reliable marker for soft tissue tumors with muscle differentiation, i.e. leiomyomas (LM), leiomyosarcomas (LMS) and rhabdomyosarcomas (RMS), for which it displayed a higher degree of sensitivity than desmin antibodies (1). This was confirmed by Schmidt, et al. (1988) (2) who found 29/30 RMS, including embryonal, alveolar, botryoid and pleomorphic subtypes, and regardless of the degree of differentiation, to be positive. A study comprising 285 well characterized soft tissue tumors found 17/17 RMS, 31/32 LMS, 23/23 LM and 3/5 pleomorphic liposarcomas to be positive (5). The majority of glomus tumors also reacted with the antibody (5, 8). Desmoid tumors showed occasional positive cells in 9/15 cases (5). Similar results were reported by others who found 34/35 RMS, 11/22 LMS, 5/6 LM and 4/4 rhabdomyomas to be positive (6). The myofibroblasts of some lesions, including reactive tissue, healing wounds and atherosclerotic plaques also stained with the antibody in the majority of cases (1, 4, 6, 9). The antibody was also used successfully for the differentiation of noninvasive (consistently actin positive) from invasive breast tumors (actin negative) (7). Nonmuscle sarcomas and neoplastic cells of carcinomas, melanomas, and lymphomas are non-reactive (1, 5).