Monoclonal Mouse Anti-Human Muscle Actin Clone HHF35

Code M0635

Intended use
For in vitro diagnostic use.

Monoclonal Mouse Anti-Human Muscle Actin, Clone HHF35, is intended for use in immunohistochemistry (IHC). Results aid in the classification of soft tissue tumors with muscle differentiation, i.e. leiomyoma (LM), leiomyosarcoma (LMS), and rhabdomyosarcoma (RMS).[2, 3] Differential classification is aided by the results from a panel of antibodies. 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. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

Summary and explanation
Actin, a highly conserved, ubiquitous cytoskeletal protein of muscle and nonmuscle cells, exists in three isotypes (α, β, γ) that differ by their amino acid sequences and isoelectric points. Monoclonal mouse anti-human Muscle Actin, clone HHF35 was made by immunizing with a polypeptide fraction of human myocardium from a case of idiopathic hypertrophic subaortic stenosis.[1]

Refer to *Dako General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures for: Principle of Procedure, Materials Required, Not Supplied, Storage, Specimen Preparation, Staining Procedure, Quality Control, Troubleshooting, Interpretation of Staining, General Limitations.

Reagent provided
Monoclonal Mouse antibody provided in liquid form as tissue culture supernatant in 0.05 mol/L Tris-HCl, pH 7.2 and 0.015 mol/L sodium azide. This product contains stabilizing protein.

Clone: HHF35[1] Isotype: IgG1, kappa

Mouse IgG concentration: See label on vial.

The protein concentration between lots may vary without influencing the optimal dilution. The titer of each individual lot is compared and adjusted to a reference lot to ensure a consistent immunohistochemical staining performance from lot-to-lot.

Immunogen
SDS extracted protein fraction of human myocardium

Specificity
Actin does not react with the α-actin of non-muscle (endothelial cells) sources.[1] Gel electrophoresis and immunoblots show the specificity of HHF35 to be for the α- and γ-actin isotypes of skeletal, cardiac and smooth muscle.[1]

Materials required, but not supplied
Refer to *Dako General Instructions for Immunohistochemical Staining* and/or the detection system instructions. Suggested diluent for IHC procedures: Dilution of this antibody in a buffer containing 0.08 mol/L EDTA is recommended to reduce nonspecific background staining.[3] The following negative control is recommended for IHC procedures: Mouse IgG1 (Code X0931).

Precautions
1. For in vitro diagnostic use.
2. For professional users.
3. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, NaN_3 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.
4. As with any product derived from biological sources, proper handling procedures should be used.
5. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
6. Unused reagents should be disposed of according to local, State, and Federal regulations.

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 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
Paraffin sections: Anti-Muscle Actin, HHF35 can be used on formalin-fixed, paraffin-embedded tissue sections. Pretreatment of deparaffinized tissue with proteolytic enzymes is not required.

Staining procedure
These are guidelines only. Optimal antibody concentrations may vary depending on specimen and preparation method, and should be validated by each individual laboratory. The performance of this antibody should be established by the user when utilized with other manual staining systems or automated platforms.

Dilution: M0635 may be used at a dilution of 1:50 when performing IHC using the LSAB2 detection system. Follow the procedure for the detection system selected.

Quality control: Positive and negative control tissues as well as negative control reagent should be run simultaneously using the same protocol as the patient specimens.

Staining interpretation
The cellular staining pattern for anti-muscle actin is cytoplasmic.

Product specific limitations
1. The addition of EDTA to an HHF35 primary antibody diluent reduced nonspecific staining[2,3] and also decreased the chances of false-positive staining of neuroblastomas, retinoblastomas, and Ewing sarcomas while maintaining adequate sensitivity for myogenic tumors.[3]
2. Mild enzyme predigestion (pepsin, pronase, trypsin) was found to improve staining quality of formalin-fixed, paraffin-embedded tissue;[4] however, Dako does not recommend proteolytic tissue pretreatment.
3. Only rarely was immunoreactivity with HHF35 observed in isolated spindle cells of the liver, lymph nodes, kidney, pancreas, and the adrenal gland.[5]
4. Neoplastic cells of some pleomorphic undifferentiated sarcomas (malignant fibrous histiocytomas, MFH) have been reported positive, localized only to the smooth muscle cells and pericytes of blood vessels.[2,4]

Performance characteristics
Normal tissues: In normal tissue, HHF35 demonstrates cytoplasmic staining of striated fibers of skeletal muscles, the smooth muscles of arteries, veins and pericytes of smaller arteries, the tunica muscularis of the GI tract, the myometrium of the uterus, prostatic stroma, the capsule cells of several parenchymal organs, including liver, kidney, lymph nodes and spleen, and the myoepithelial layers of the mammary ducts and glands, and the eccrine sweat, bronchial and salivary glands.[1,2,4-6] Other non-muscle cells are non-reactive, including vascular endothelial cells, epithelial cells, lymphoid cells, macrophages, connective tissue, and neural cells.[1,2,5,6]

Abnormal tissues: In pathological tissues, HHF35 labeled soft tissue tumors with muscle differentiation, i.e. leiomyomas (LM), leiomyosarcomas (LMS) and rhabdomyosarcomas (RMS).[2] This was confirmed by Schmidt, et al.[3] who found 29/30 RMS, including embryonal, alveolar, botryoid and pleomorphic subtypes, regardless of the degree of differentiation, to be labeled. A study comprising 285 well characterized soft tissue tumors[5] found 17/17 RMS, 31/32 LMS, 23/23 LM and 3/5 pleomorphic liposarcomas to be immunoreactive with HHF35. The majority of glomus tumors also reacted with HHF35.[4,7] Desmoid tumors showed occasional labeled cells in 9/15 cases.[4] Similar results were reported by others[8] who found 34/35 RMS, 11/22 LMS, 5/6 LM and 4/4 rhabdomyomas to be HHF35 reactive. The myofibroblasts of some lesions, including reactive tissue, healing wounds and atherosclerotic plaques also stained with HHF35 in the majority of cases.[1,2,5,8] HHF35 also labeled noninvasive breast tumors while invasive breast tumors were not labeled.[6] Non-muscle sarcomas and neoplastic cells of carcinomas, melanomas, and lymphomas are non-reactive.[2,4]

References

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Explanation of symbols

REF	Catalogue number		Temperature limitation	IVD	In vitro diagnostic medical device
	Manufacturer	LOT	Batch code		Contains sufficient for <n> tests
	Use by		Consult instructions for use	EC REP	Authorized representative in the European Community

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