NAME: Monoclonal Mouse Anti-Human Muscle Actin, Clone HHF35

IMMUNOGEN: SDS extracted protein fraction of human myocardium

CLONE/REF: HHF35¹ ²

CLASS/SUBCLASS: IgG₁, kappa

CODE NO.: M0635

Concentrated antibody

Lot No. 101

IgG Conc.: 106 µg/mL (Refractometry)

Total Protein Conc.: 10.7 mg/mL (Refractometry)

INTENDED USE:

This antibody is intended for laboratory use to identify qualitatively by light microscopy an epitope present on muscle actin in normal and neoplastic tissues using immunohistochemical (IHC) test methods. Clone HHF35 has been demonstrated to be a reliable marker for soft tissue tumors with muscle differentiation, i.e. leiomyomas (LM), leiomyosarcomas (LMS), and rhabdomyosarcomas (RMS).¹² 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.

Refer to the General Instructions for immunohistochemistry (IHC) or in the Detection System Instructions of IHC procedures for:


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. The monoclonal mouse anti-human Muscle Actin, clone HHF35 was made by immunizing mice with a polypeptide fraction of human myocardium from a case of idiopathic hypertrophic subaortic stenosis. It 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

REAGENTS PROVIDED:

Anti-Muscle Actin is provided in a 1.0 mL size as tissue culture supernatant dialyzed against 0.05 mol/L Tris-HCl, pH 7.2, and 0.015 mol/L sodium azide. Contains stabilizing protein.

M0635 may be used at a dilution of 1:50 when performing IHC using the DAKO LSAB² detection system. These are guidelines only. Optimal antibody concentrations may vary depending on specimen and preparation method, and should be determined by each individual laboratory.

MATERIALS REQUIRED, NOT SUPPLIED:

Refer to the General Instructions for IHC and/or the Detection System Instructions.

Suggested diluent for IHC procedures: Dilution of this antibody in a buffer containing 80 mmol/L EDTA is recommended to reduce nonspecific background staining.³ The following negative control is recommended for IHC procedures: Mouse IgG₁ (DAKO⁶ Code No. X0931).

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, build-ups of NaN₃ may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing.⁴ ⁵

3. Minimize microbial contamination of reagents or an increase in nonspecific staining may occur.

STORAGE:

Store at 2-8°C or -20°C in single-use aliquots.

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.⁶
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:* Anti-Muscle Actin, HHF35 can be used on formalin-fixed, paraffin-embedded tissue sections. Pretreatment of tissue with proteolytic enzymes is not required.

*Cryostat Sections And Cell Smears:* Anti-Muscle Actin, HHF35 can be used for labelling acetone-fixed cryostat sections or fixed cell smears.

**STAINING PROCEDURE:**
Follow the procedure for the detection system selected.

**PRODUCT SPECIFIC LIMITATIONS:**
1. Schmidt, et al. (1988) and others found the addition of EDTA to an HHF35 primary antibody diluent reduced nonspecific staining and also decreased the chances of false-positive staining of neuroblastosmas, retinoblastomas, and Ewing Sarcomas while maintaining adequate sensitivity for myogenic tumors.  
2. Miettinen (1988) found mild enzyme predigestion (pepsin, pronase, trypsin) to improve staining quality of formalin-fixed, paraffin-embedded tissue, however DAKO does not recommend 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.  
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.

**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. Other non-muscle cells are non-reactive, including vascular endothelial cells, epithelial cells, lymphoid cells, macrophages, connective tissue, and neural cells.  

*Abnormal tissues:* In pathological tissues, HHF35 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. This was confirmed by Schmidt, et al. (1988) who found 29/30 RMS, including embryonal, alveolar, botryoid and pleomorphic subtypes, and regardless of the degree of differentiation, to be HHF35 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 immunoreactive with HHF35. The majority of glomus tumors also reacted with HHF35. Desmoid tumors showed occasional positive cells in 9/15 cases. Similar results were reported by others 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. HHF35 was also used successfully for the differentiation of noninvasive (consistently actin positive) from invasive breast tumors (actin negative).

Non-muscle sarcomas and neoplastic cells of carcinomas, melanomas, and lymphomas are non-reactive.

**REFERENCES:**
7. Miettinen M. Antibody specific to muscle actins in the diagnosis and classification of soft tissue tumors. Amer J Pathol 1988; 130:205