303341EFG 001

Dako

CALPONIN

CE

Monoclonal Mouse Anti-Human Calponin Clone CALP

ENGLISH
Code M3556

Intended use
For In Vitro Diagnostic Use.

Summary and explanation
Calponin is a calmodulin, F-actin and tropomyosin binding protein which is thought to be involved in the regulation of smooth muscle contraction.[2,3] Calponin expression is restricted to smooth muscle cells and has been shown to be a marker of the differentiated (contractile) phenotype of developing smooth muscle.[2-5]

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
Anti-human calponin, CALP is a mouse monoclonal antibody supplied in liquid form as tissue culture supernatant (containing fetal bovine serum) dialyzed against 0.05 mol/L Tris-HCl, pH 7.2 and 0.015 mol/L sodium azide.

Clone: CALP[1] Isotype: IgG_1, kappa
Mouse IgG concentration mg/L: See label on vial.

Anti-calponin, CALP may be used at a dilution of 1:50 in the LSAB method determined on enzyme digested formalin-fixed, paraffin-embedded tissue. These are guidelines only; optimal dilutions should be determined by the individual laboratory.

Immunogen
Crude human uterus extract[1]

Specificity
Multiple isoelectric variants of calponin have been identified, however only two molecular weight isoforms exist; a 34 kD form and a 29 kD form.[5] Expression of the 29 kD form, *l*-calponin, is primarily restricted to muscle of the urogenital tract, whereas the higher molecular weight variant has been demonstrated in vascular and visceral smooth muscle.[2-5] In Western blotting, monoclonal anti-calponin, CALP, has been demonstrated to react with only the 34 kD form of calponin in extracts of human aortic medial smooth muscle and is unreactive with fibroblast extracts of cultivated human foreskin.[1]

Materials required, but not supplied
Refer to Dako's *General Instructions for Immunohistochemical Staining* and/or the detection system instructions.

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, 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.
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 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-calponin, CALP can be used on formalin-fixed, paraffin-embedded tissue sections. Pretreatment of tissue with proteolytic enzymes should be performed prior to staining.

For improved staining results, the deparaffinized tissue sections may be treated with a proteolytic enzyme followed by target retrieval. For greater adherence of tissue sections to glass slides, the use of Silanized Slides (code S3003) is recommended. Deparaffinized tissue sections should first be treated for 5 to 10 minutes with a mild enzyme solution. A recommended proteolytic enzyme is Proteinase K (code S3004) which must be further diluted 1:500 in 0.05 mol/L Tris-HCl, pH 7.6 to give a final concentration of 0.04 mg/mL.

Following proteolytic digestion, tissue sections can be treated with heat. When using the water bath method, preheat a Coplin jar containing 0.01 mol/L citrate buffer, pH 6.0 as well as a water bath to 95–99 °C. When the temperature has stabilized, place tissue sections into the Coplin jar containing the preheated buffer. Heat the tissue sections for 40 minutes. For improved staining results and a shorter incubation time, Target Retrieval Solution (code S1700) can be used in place of the 0.01 mol/L citrate buffer. Under these conditions the incubation time in the water bath may be reduced to 20 minutes. After thermal treatment, allow the jar with buffer and slides to cool for 20 minutes at room temperature. Rinse well with distilled water and place slides into buffer.

Cryostat Sections and Cell Smears
Anti-calponin, CALP can also be used to label cryostat sections or cell smears.

Staining procedure
Follow the recommended procedure for the detection system selected.

Staining interpretation
The staining pattern for anti-calponin is cytoplasmic.

Performance characteristics
Normal Cells
In immunohistochemical (IHC) studies on cryostat sections of human fetal tissue, monoclonal anti-calponin, CALP, was found to stain developing visceral smooth muscle of trachea, jejenum, esophagus and uterus in 10 and 20 week-old fetuses. Monoclonal anti-calponin did not react positively with 10 and 20 week-old fetal aortic smooth muscle cells.[1] Monoclonal antibody CALP was found to localize calponin in cryostat sections of adult visceral and vascular smooth muscle but not in epithelial cells, endothelial cells, or connective tissue fibroblasts. Adult aortic cells of the tunica media and a portion of subendothelial intimal cells were found to stain positively.[1] In cryostat sections and routinely fixed specimens of normal human breast, calponin expression has been demonstrated in smooth muscle cells of blood vessels and myoepithelial cells in the lobules, ducts and galactophorous sinuses.[6,7] Periacinar and periductal myoepithelial cells of the salivary gland have also been shown to react positively with anti-calponin, whereas ductal epithelial cells were negative.[8]

Tumor Cells
Calponin expression has been demonstrated by IHC in myoepithelial cells in benign and malignant breast lesions.[6,7] Myoepithelial cells in papillomas of the breast were found to stain positively with anti-calponin but no reactivity was observed in intracystic papillary carcinomas. In ductal carcinoma *in situ,* calponin immuno-reactivity has been demonstrated in myoepithelial cells at the periphery of involved ducts and lobules in complex sclerosing lesions of the breast.[7] Anti-calponin was shown to label stromal myofibroblasts in the majority of invasive breast carcinomas tested but was unreactive with tumor cells.[6,7] Reactivity has also been observed in neoplastic myoepithelium of routinely fixed

Leistungseigenschaften

Normale Zellen

In immunhistochemischen (IHC) Studien mit Kryostatschnitten aus humanem fetalen Gewebe zeigte sich, dass Anti-Calponion, CALP, viszerale glatte Muskeln der Trachea, des Jejunums, Ösophagus und Uterus während deren Entwicklung in 10 und 20 Wochen alten Föten anfärbt. Monoklonales Anti-Calponin reagierte mit glatten Muskeln der Aorta von 10 und 20 Wochen alten Föten nicht positiv.[1] Der monoklonale Antikörper CALP lokalisierte Calponin in Kryostatschnitten aus glatten Muskeln der Gefäße und Viszera Erwachsener, nicht jedoch in Epithelzellen, Endothelialzellen oder in Fibroblasten des Bindegewebes. Bei Erwachsenen erfolgte eine positive Färbung von Aortazellen der Tunica media und einem Teil von subendothelialen Intimazellen.[1] In Kryostatschnitten und routinemäßig fixierten Proben aus normalem menschlichem Brustgewebe wurde eine Expression von Calponin in glatten Muskelzellen von Blutgefäßen und myoepithelialen Zellen in den Lobuli, den Gängen und den Milchsäckchen nachgewiesen.[6,7] Periazinäre und periduktale Myoepithelzellen der Speicheldrüsen reagieren ebenfalls mit Anti-Calponin positiv. Duktale Epithelzellen waren demgegenüber negativ.[8]

Tumorzellen

Mithilfe von IHC wurde die Expression von Calponin in myoepithelialen Zellen benigner und maligner Brustläsionen nachgewiesen.[6,7] Dabei färbten sich myoepitheliale Zellen in Brustpapillomen mit Anti-Calponin positiv, in intrazystischen papillären Karzinomen wurde jedoch keine Reaktivität beobachtet. Im duktalen Karzinom *in situ* zeigte Calponin in myoepithelialen Zellen an der Peripherie beteiligter Gänge sowie in Lobuli komplexer sklerosierender Läsionen der Brust eine Immunreaktivität.[7] Anti-Calponin wies bei der Mehrheit der getesteten invasiven Brustkarzinome eine Färbung stromaler Myofibroblasten auf, war jedoch mit Tumorzellen nicht reaktiv.[6,7] Eine Reaktivität wurde ebenso im neoplastischen Myoepithel routinemäßig fixierter, pleomorpher Adenome der Speicheldrüse nachgewiesen.[8]

References
Références
Literatur

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REF Catalogue number Référence du catalogue Bestellnummer	Temperature limitation Limites de température Zulässiger Temperaturbereich	Consult instructions for use *Consulter les instructions d'utilisation* *Gebrauchsanweisung beachten*
Manufacturer Fabricant Hersteller	LOT Batch code Code du lot Chargenbezeichnung	Use by Utiliser jusque Verwendbar bis
EC REP Authorized representative in the European Community Représentant Autorisé dans la Communauté Européenne Autorisierter Repräsentant in der EU		IVD In vitro diagnostic medical device Dispositif médical de diagnostic in vitro In-vitro-Diagnostikum

P10039/Rev C

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