MARCKS Mouse Monoclonal Antibody

MCA-5F9

Ordering Information
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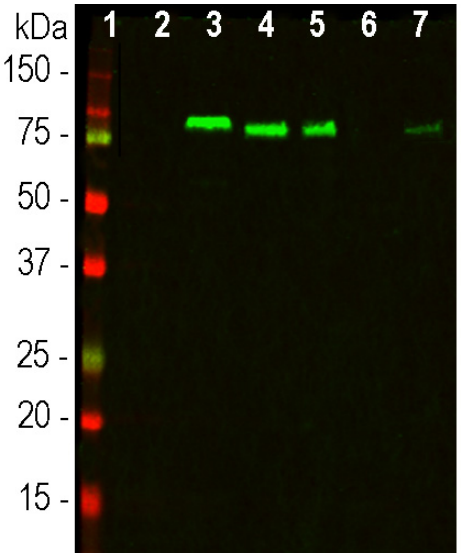
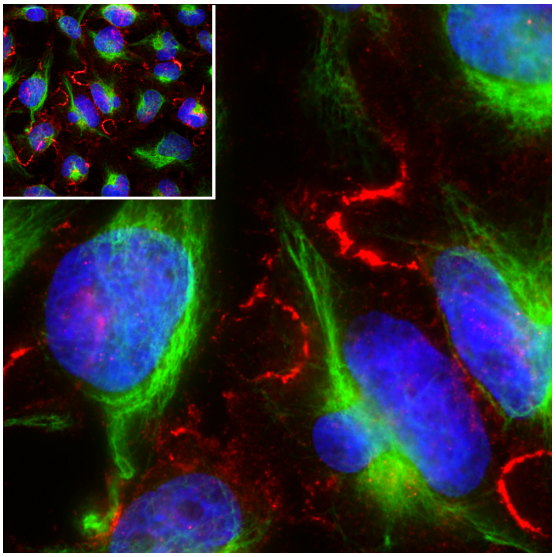
HGNC Name: *MARCKS*
UniProt: *P29966*
RRID: *AB_2744535*
Immunogen: *Full length human MARCKS expressed in and purified from E. coli.*
Format: *Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN_3*
Storage: *Store at 4°C for short term, for longer term at -20°C*
Recommended dilutions:
WB: 1:2,000. IF/ICC and IHC: 1:2,000

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC, IHC	Mouse	IgG1 heavy, κ light	~80kDa by SDS-PAGE	Hu, Mo



Western blot analysis of different cell lysates using mouse mAb to MARCKs, MCA-5F9, in green: [1] protein standard, [2] NIH-3T3, [3] HEK293, [4] HeLa, [5] SH-SY5Y, [6] C6 and [7] COS1 cells. The band at about 80kDa corresponds to the MARCKS protein detected only in the human and monkey cells. The antibody does not recognize the rodent form of the MARCKS protein.



Immunofluorescent analysis of HeLa cell culture stained with mouse mAb to MARCKS, MCA-5F9, dilution 1:1,000 in red, and costained with chicken pAb to vimentin, CPCA-Vim, dilution 1:10,000 in green. The blue is Hoechst staining of nuclear DNA. MARCKS antibody binds to MARCKS protein expressed in the membrane structures and in cytoplasm, while the vimentin antibody stains the intermediate filaments.

Background:

Myristolyated alanine rich C-kinase substrate, hence MARCKS, was originally discovered by as a major substrate for protein kinase C in the brain and other tissues (1). The protein sequence is indeed unusually rich in alanine, the human sequence containing 30.7% alanine, though it is also unusually rich in glutamic acid (16.0%), proline (10.5%) and glycine (10.2%). The sequence has almost no hydrophobic amino acids and has a calculated molecular weight of 31.4kDa and PI of 4.46. The unusual composition is responsible for the low SDS-PAGE mobility, which gives an apparent molecular weight of 80kDa. MARCKS belongs to the family of intrinsically unstructured proteins (IUP) which typically have a high content of glutamic acid and proline and low hydrophobic content (5). IUP proteins or regions with these properties within larger proteins hove no defined structure but adopt a defined conformation on binding to their ligands. MARCKS interacts with actin and calcium in a manner regulated by protein kinase C (2-4). It is a major protein of the brain concentrated in the synapses of neurons and is membrane localized due to the N-terminal lipid myristoyl group. Antibody to MARCKS can therefore be used as a marker of synaptic regions. Genetic knock out of MARCKS in transgenic mice is perinatal lethal and associated with aberrant brain development suggesting a fundamental importance in the CNS (6). Since the MARCKS protein sequence is relatively poorly conserved across species boundaries, antibodies to the human protein may not work well on rodent tissues, which is the case with this reagent.

The MCA-5F9 antibody was made against recombinant full length human MARCKS expressed in and purified from *E. coli*. The antibody works well on human cells and tissues but is not recommended for work on rodent material. The antibody works well for western blotting and for IF, ICC and IHC (see data under "Additional Info" tab). We also supply rabbit and a chicken polyclonal antibodies to this protein, RPCA-MARCKS and CPCA-MARCKS.

References:

1. *Hirai M, Shimizu N. Purification of two distinct proteins of approximate Mr 80,000 from human epithelial cells and identification as proper substrates for protein kinase C. Biochem. J. 270:583-9 (1990).*
2. *Hartwig JH, et al. MARCKS is an actin filament crosslinking protein regulated by protein kinase C and calcium-calmodulin. Nature 356:618-22 (1992).*
3. *Aderem A. The MARCKS Brothers: A Family of Protein Kinase C Substrates. Cell 71:713-6 (1992).*
4. *Blackshear PJ. The MARCKS family of cellular protein kinase C substrates. J. Biol. Chem. 268:1501-4 (1993).*
5. *Tompa P. Intrinsically unstructured proteins. Trends Biochem. Sci. 27:527-33 (2002).*
6. *Stumpo DJ, Bock CB, Tuttle JS, Blackshear PJ. MARCKS deficiency in mice leads to abnormal brain development and perinatal death. PNAS 92:944-8 (1995).*

Abbreviation Key:

mAb—Monoclonal Antibody pAb—Polyclonal Antibody WB—Western Blot IF—Immunofluorescence ICC—Immunocytochemistry IHC—Immunohistochemistry E—ELISA Hu—Human Mo—Monkey Do—Dog Rt—Rat Ms—Mouse Co—Cow Pi—Pig Ho—Horse Ch—Chicken Dr—D. rerio Dm—D. melanogaster Sm—S. mutans Ce—C. elegans Sc—S. cerevisiae Sa—S. aureus Ec—E. coli.