MARCKS Rabbit Polyclonal Antibody

RPCA-MARCKS

Ordering Information
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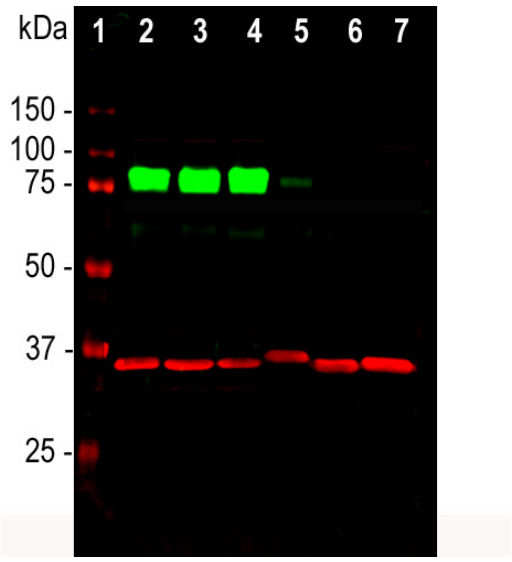
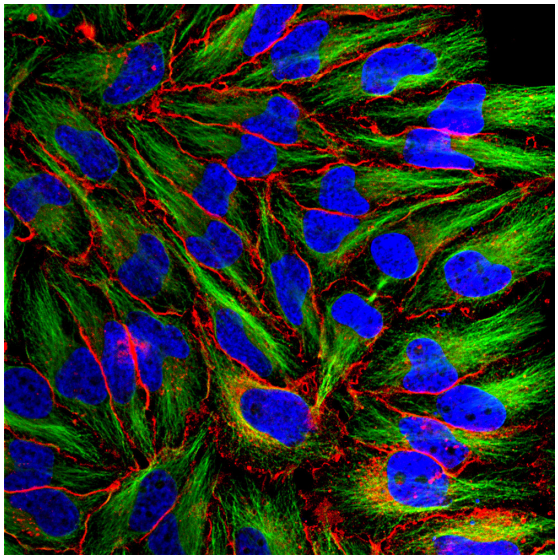
HGNC Name: *MARCKS*
UniProt: *P29966*
RRID: *AB_2250333*
Immunogen: *Full length recombinant human MARCKS expressed in and purified from E. coli*
Format: *Affinity purified with 50% PBS and 50% glycerol plus 5mM NaN_3*
Storage: *Store at 4°C for short term. For longer term, store at -20°C.*
Recommended dilutions:
WB: 1:10,000-1:20,000. IF/ICC and IHC: 1:500-1:1,000.

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC	Rabbit		80kDa by SDS-PAGE	Hu



Western blot analysis of cell line lysates probed simultaneously with rabbit pAb to MARCKS, RPCA-MARCKS, dilution 1:1,000, in green and mouse mAb to GAPDH, MCA-1D4, dilution 1:5,000 in red: [1] protein standard (red), [2] HEK293, [3] HeLa, [4] SH-SY5Y, [5] COS1, [6] NIH-3T3, [7] C6 cells. The strong band at ~80kDa corresponds to MARCKS protein, detected only in the proteins of human origin. Slight reactivity is observed in the monkey cells [5], but no reactivity is seen on rodent cells, [6] and [7]. GAPDH antibody used as a loading control revealing a single band at ~37kDa in all preparations.



Immunofluorescent analysis of HeLa cells stained with rabbit pAb to MARCKS, RPCA-MARCKS, dilution 1:1,000 in red, and costained with mouse mAb to β-tubulin, MCA-1B12, dilution 1:10,000 in green. The blue is DAPI staining of nuclear DNA. The RPCA-MARCKS antibody recognizes protein localized in the plasma membrane and cytoplasm, while the β-tubulin antibody stains the network of cytoplasmic microtubules.

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. It is also 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. These unusual features are 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 RPCA-MARCKS 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. We also supply a mouse monoclonal and a chicken polyclonal antibodies to this protein, MCA-5F9 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).

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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.