

Calmodulin Monoclonal Antibody (2D1)

Catalog Number MA3-917

Product data sheet

Details		Species Reactivity	
Size	200 µL	Species reactivity	Bacteria, Bovine, Chicken, Human, Mouse, Rat
Host/Isotope	Mouse / IgG1	Published species	Fungi, Algae, Rat, Shrew, Plant, Mouse, Human, Not Applicable
Class	Monoclonal	Tested Applications	
Type	Antibody	Dilution *	
Clone	2D1	ELISA (ELISA)	Assay-dependent
Immunogen	Calmodulin purified from Dictyostelium discoideum.	Flow Cytometry (Flow)	2 µg / 10^6 cells
Conjugate	Unconjugated	Immunohistochemistry (IHC)	1:20
Form	Liquid	Immunohistochemistry (Paraffin) (IHC (P))	1:20
Concentration	1 mg/mL	Western Blot (WB)	1:500
Purification	Protein A	Immunocytochemistry (ICC/IF)	1:50
Storage buffer	PBS	Published Applications	
Contains	0.05% sodium azide	Immunoprecipitation (IP)	See 1 publications below
Storage Conditions	-20° C, Avoid Freeze/Thaw Cycles	Western Blot (WB)	See 4 publications below
		Immunohistochemistry (IHC)	See 2 publications below
		Neutralization (Neu)	See 1 publications below
		Immunocytochemistry (ICC/IF)	See 1 publications below

* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Product specific information

MA3-917 detects calmodulin from human, bovine, chicken, Chlamydomonas, Dictyostelium, mouse and rat samples. This antibody does not detect parvalbumin, tropinin, S-100, or myosin light chain kinase (MLCK). MA3-917 has been successfully used in Western blot, immunocytochemistry and ELISA procedures. By Western blot, this antibody detects a 17 kDa protein representing calmodulin from Dictyostelium cell lysate. Addition of EGTA to buffers completely inhibits antibody staining. Immunohistochemical staining of calmodulin in Dictyostelium cells with MA3-917 results in staining of the contractile vacuoles. The MA3-917 antigen is calmodulin purified from Dictostelium discoideum.

Background/Target Information

Calmodulin mediates the control of a large number of enzymes, ion channels, aquaporins and other proteins by Ca2+. Among the enzymes to be stimulated by the calmodulin-Ca2+ complex are a number of protein kinases and phosphatases. Together with CCP110 and centrin, is involved in a genetic pathway that regulates the centrosome cycle and progression through cytokinesis. Calmodulin is a small, highly conserved calcium binding protein found in all eukaryotic cells. With the capacity to bind up to four calcium ions, this 17 kDa protein acts as an important intracellular receptor for regulatory calcium signals. As it binds calcium, calmodulin undergoes conformational changes which can increase its affinity for target proteins. It acts both directly, through interaction with key target enzymes, and indirectly, via specific kinases. Studies have found that calmodulin participates in the regulation of several biological processes including energy and biosynthetic metabolism, cell motility, exocytosis, cytoskeletal assembly, and intracellular modulation of both cAMP and calcium concentrations. Calmodulin is the archetype of the family of calcium-modulated proteins of which nearly 20 members have been found. They are identified by their occurrence in the cytosol or on membranes facing the cytosol and by a high affinity for calcium. Calmodulin contains 149 amino acids and has 4 calcium-binding domains. Its functions include roles in growth and the cell cycle as well as in signal transduction and the synthesis and release of neurotransmitters.

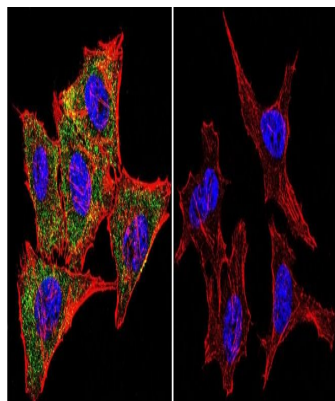
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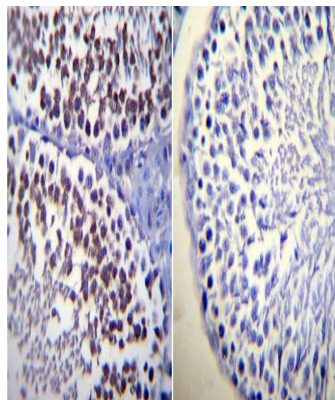
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Product Images For Calmodulin Monoclonal Antibody (2D1)



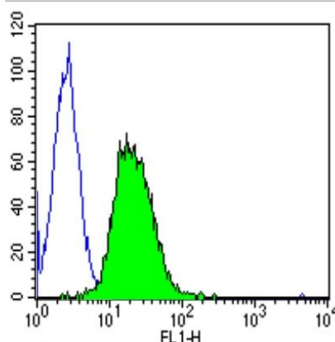
Calmodulin Antibody (MA3-917) in ICC/IF

Immunofluorescent analysis of Calmodulin using Calmodulin Monoclonal antibody (2D1) (Product # MA3-917) shows staining in HeLa cells. Calmodulin staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with or an antibody recognizing Calmodulin (Product # MA3-917) at a dilution of 1:20 over night at 4 °C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody (Product # 35552 for GAR, Product # 35503 for GAM). Images were taken at 60X magnification.



Calmodulin Antibody (MA3-917) in IHC (P)

Immunohistochemistry was performed on normal biopsies of deparaffinized Rat testis tissue. To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer, microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:20 with a mouse monoclonal antibody recognizing Calmodulin (Product # MA3-917) or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP, followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.

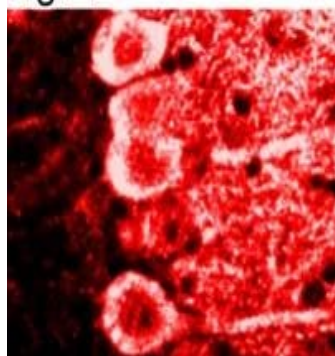


Calmodulin Antibody (MA3-917) in Flow

Flow cytometry analysis of Calmodulin in C6 cells compared to an isotype control (blue). Cells were harvested, adjusted to a concentration of $1-5 \times 10^6$ cells/mL, fixed with 2% paraformaldehyde and washed with PBS. Cells were penetrated by dropping the supernatant, adding 90% methanol and incubated for 10 minutes at room temperature. Following penetration, cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with a Calmodulin monoclonal antibody (Product # MA3-917) at a dilution of 2 µg/test for 60 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated goat anti-mouse IgG (H+L) secondary antibody and re-suspended in PBS for FACS analysis.

Cell: C6
Concentration: 2µg/test (100µl)
Theory location : Cytoplasm

Fig. 1



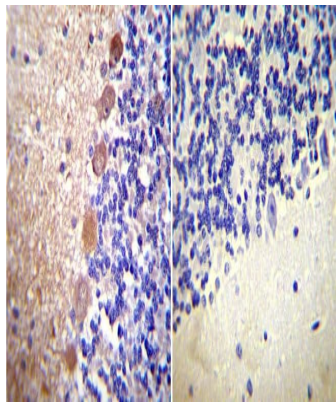
Calmodulin Antibody (MA3-917) in IHC

Immunolocalization of calmodulin in rat brain using Product # MA3-917.

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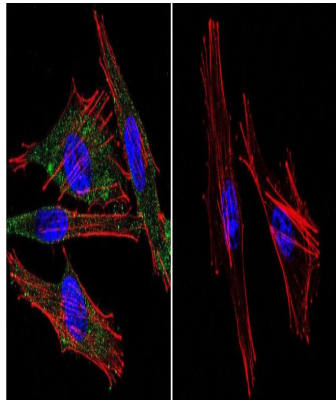
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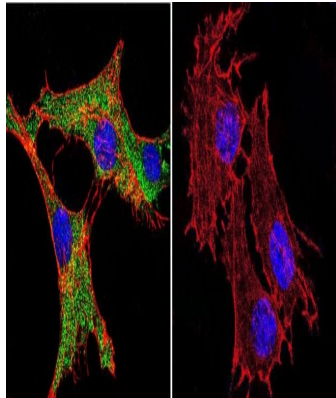
Calmodulin Antibody (MA3-917) in IHC (P)

Immunohistochemistry was performed on normal biopsies of deparaffinized Rat cerebellum tissue. To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer, microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:20 with a mouse monoclonal antibody recognizing Calmodulin (Product # MA3-917) or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP, followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



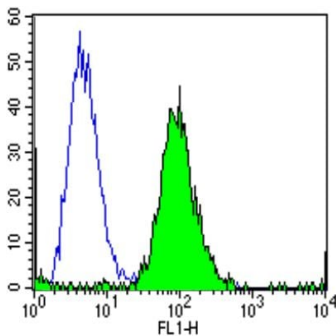
Calmodulin Antibody (MA3-917) in ICC/IF

Immunofluorescent analysis of Calmodulin using Calmodulin Monoclonal antibody (2D1) (Product # MA3-917) shows staining in A2058 melanoma cells. Calmodulin staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with or an antibody recognizing Calmodulin (Product # MA3-917) at a dilution of 1:20 over night at 4 °C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody (Product # 35552 for GAR, Product # 35503 for GAM). Images were taken at 60X magnification.



Calmodulin Antibody (MA3-917) in ICC/IF

Immunofluorescent analysis of Calmodulin using Calmodulin Monoclonal antibody (2D1) (Product # MA3-917) shows staining in C6 glioma cells. Calmodulin staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with or an antibody recognizing Calmodulin (Product # MA3-917) at a dilution of 1:20 over night at 4 °C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody (Product # 35552 for GAR, Product # 35503 for GAM). Images were taken at 60X magnification.



Calmodulin Antibody (MA3-917) in Flow

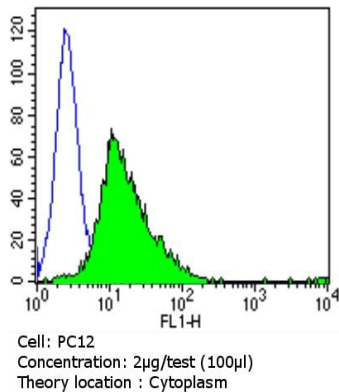
Flow cytometry analysis of Calmodulin in MCF-7 cells compared to an isotype control (blue). Cells were harvested, adjusted to a concentration of $1-5 \times 10^6$ cells/mL, fixed with 2% paraformaldehyde and washed with PBS. Cells were penetrated by dropping the supernatant, adding 90% methanol and incubated for 10 minutes at room temperature. Following penetration, cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with a Calmodulin monoclonal antibody (Product # MA3-917) at a dilution of 2 µg/test for 60 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a DyLight 488-conjugated goat anti-mouse IgG (H+L) secondary antibody and re-suspended in PBS for FACS analysis.

Cell: MCF-7
Concentration: 2µg/test (100µl)
Theory location : Cytoplasm

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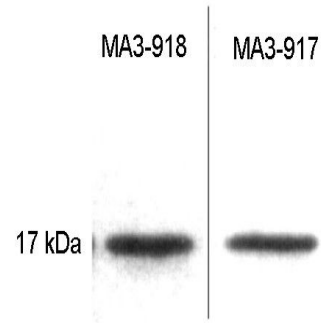
Calmodulin Antibody (MA3-917) in Flow

Flow cytometry analysis of Calmodulin in PC12 cells compared to an isotype control (blue). Cells were harvested, adjusted to a concentration of $1-5 \times 10^6$ cells/mL, fixed with 2% paraformaldehyde and washed with PBS. Cells were penetrated by dropping the supernatant, adding 90% methanol and incubated for 10 minutes at room temperature. Following penetration, cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with a Calmodulin monoclonal antibody (Product # MA3-917) at a dilution of 2 µg/test for 60 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated goat anti-mouse IgG (H+L) secondary antibody and re-suspended in PBS for FACS analysis.

Fig. 2

Calmodulin Antibody (MA3-917) in WB

Western blot of purified calmodulin using Product # MA3-917 and Product # MA3-918.



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PubMed References For Calmodulin Monoclonal Antibody (2D1)

1 Immunoprecipitation References

Species / Dilution	Summary
	MA3-917 was used in Immunoprecipitation to conclude that the neuronal ECM can be remodeled frequently through mechanisms that involve endocytosis and recycling of ECM proteins.
Mouse / 1:100	Nature communications (2021; 12:) "Extracellular matrix remodeling through endocytosis and resurfacing of Tenascin-R." Author(s):Dankovich TM,Kaushik R,Olsthoorn LHM,Petersen GC,Giro PE,Kluever V,Agüi-Gonzalez P,Grewe K,Bao G,Beuermann S,Hadi HA,Doeren J,Klöppner S,Cooper BH,Dityatev A,Rizzoli SO PubMed Article URL: http://dx.doi.org/10.1038/s41467-021-27462-7

4 Western Blot References

Species / Dilution	Summary
	MA3-917 was used in western blot to analyze Drosophila pericentrin and its requirement for interaction with calmodulin for function at centrosomes and neuronal basal bodies but not sperm basal bodies
Not Applicable / 1:500	Molecular biology of the cell (2014; 25: 2682) "Drosophila pericentrin requires interaction with calmodulin for its function at centrosomes and neuronal basal bodies but not at sperm basal bodies." Author(s):Galletta BJ,Guillen RX,Fagerstrom CJ,Brownlee CW,Lerit DA,Megraw TL,Rogers GC,Rusan NM PubMed Article URL: http://dx.doi.org/10.1091/mbc.E13-10-0617
Plant / Not Cited	MA3-917 was used in western blot to study the mechanism for the effect of oligogalacturonide on Arabidopsis thaliana cultured cells
	Plant biology (Stuttgart, Germany) (2004; 6: 192) "Involvement of the plasma membrane Ca2+-ATPase in the short-term response of Arabidopsis thaliana cultured cells to oligogalacturonides." Author(s):Romani G,Bonza MC,Filippini I,Cerana M,Beffagna N,De Michelis MI PubMed Article URL: http://dx.doi.org/10.1055/s-2004-817848
Mouse / Not Cited	MA3-917 was used in Western Blotting to show that calcium-CnA signaling is hyperactivated in DM1 muscle and that such hyperactivation represents a beneficial compensatory adaptation to the disease.
	Human molecular genetics (2017; 26: 2192) "Misregulation of calcium-handling proteins promotes hyperactivation of calcineurin-NFAT signaling in skeletal muscle of DM1 mice." Author(s):Ravel-Chapuis A,Bélanger G,Côté J,Michel RN,Jasmin BJ PubMed Article URL: http://dx.doi.org/10.1093/hmg/ddx109
Algae / 1:500	MA3-917 was used in western blot to investigate the biochemical properties of centrin purified from Tetraselmis striata
	The Journal of protozoology (1992; 39: 385) "Characterization of the calcium-binding contractile protein centrin from Tetraselmis striata (Pleurostrophyceae)." Author(s):Coling DE,Salisbury JL PubMed Article URL: http://dx.doi.org/10.1111/j.1550-7408.1992.tb01468.x

2 Immunohistochemistry References

Species / Dilution	Summary
	MA3-917 was used in immunohistochemistry to study the mechanism for serotonin 5-HT3 receptor-mediated vomiting
Shrew / 1:100	PloS one (2016; 9:) "Serotonin 5-HT3 receptor-mediated vomiting occurs via the activation of Ca2+/CaMKII-dependent ERK1/2 signaling in the least shrew (Cryptotis parva)." Author(s):Zhong W,Hutchinson TE,Chebolu S,Darmani NA PubMed Article URL: http://dx.doi.org/10.1371/journal.pone.0104718
	MA3-917 was used in Immunohistochemistry to investigate the expression of calpain-1, calpain-2, calpastatin and calmodulin in gastric cancer and uninvolved gastric mucosa tissues with immunohistochemistry.
Human / 1:20	Oncology letters (2017; 14: 3705) "Comparison of the protein expression of calpain-1, calpain-2, calpastatin and calmodulin between gastric cancer and normal gastric mucosa." Author(s):Liu B,Zhou Y,Lu D,Liu Y,Zhang SQ,Xu Y,Li W,Gu X PubMed Article URL: http://dx.doi.org/10.3892/ol.2017.6617

1 Neutralization References

Species / Dilution	Summary
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MA3-917 was used in blocking or activating experiment to investigate anit-Yo antibodies in Purkinje cell death

PloS one (2016; 10:)
"Anti-Yo antibody uptake and interaction with its intracellular target antigen causes Purkinje cell death in rat cerebellar slice cultures: a possible mechanism for paraneoplastic cerebellar degeneration in humans with gynecological or breast cancers."
Author(s):Greenlee JE,Clawson SA,Hill KE,Wood B,Clardy SL,Tsunoda I,Carlson NG
PubMed Article URL:<http://dx.doi.org/10.1371/journal.pone.0123446>

1 Immunocytochemistry References

Species / Dilution	Summary
	MA3-917 was used in immunocytochemistry to investigate the interaction between calmodulin and the contractile vacuole complex during the mitosis of Dictyostelium discoideum

Fungi / Not Cited	Journal of cell science (1993; 104 (Pt 4): 1119) "Calmodulin and the contractile vacuole complex in mitotic cells of Dictyostelium discoideum." Author(s):Zhu Q,Liu T,Clarke M PubMed Article URL: http://dx.doi.org/10.1242/jcs.104.4.1119
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