





Mu-Calpain Monoclonal Antibody (9A4H8D3)

Catalog Number MA3-940 Product data sheet

Details	
Size	100 µg
Host/Isotope	Mouse / IgG1
Class	Monoclonal
Туре	Antibody
Clone	9A4H8D3
Immunogen	Purified bovine skeletal muscle 80 kDa mu-calpain subunit.
Conjugate	Unconjugated
Form	Liquid
Concentration	1.0 mg/mL
Purification	Protein A
Storage buffer	PBS with 1mg/mL BSA
Contains	0.05% sodium azide
Storage Conditions	-20° C, Avoid Freeze/Thaw Cycles

Species Reactivity	
Species reactivity	Bovine, Hamster, Human, Mouse, Pig, Rabbit, Rat
Published species	Rabbit, Pig, Rat, Yeast, Bovine, Sheep, Arthropod, Hamster, Fish, Mouse, Human, Not Applicable
Tested Applications	Dilution *
Immunohistochemistry (Frozen) (IHC (F))	Assay-dependent
Immunohistochemistry (Paraffin) (IHC (P))	Assay-Dependent
Western Blot (WB)	2 μg/mL
Immunocytochemistry (ICC/IF)	Assay-dependent
Published Applications	
Western Blot (WB)	See 38 publications below
Immunocytochemistry (ICC/IF)	See 2 publications below
Immunohistochemistry (IHC)	See 2 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

MA3-940 detects mu-calpain from human platelets and erythrocytes, bovine platelets, heart and skeletal muscle, rat myoblasts, kidney, liver and spleen, mouse lung, pig cultured cells and hamster and rabbit samples. This antibody does not cross-react with m-calpain, n-calpain, calmodulin or calpastatin. MA3-940 has been successfully used in Western blot, immunofluorescence, immunohistochemistry, and immunocytochemistry procedures. By Western blot, this antibody detects an 80 kDa protein representing mu-calpain from human platelets and erythrocytes and HeLa and A431 cell lysates. Immunocytochemical staining of mu-calpain in LLC-PK1 cells with MA3-940 results in diffuse cytoplasmic staining. This product has not been shown to be effective in immunoprecipitation experiments. The MA3-940 antigen is purified bovine skeletal muscle 80 kDa mu-calpain subunit. This antibody recognizes an epitope between amino acids 465-520 (domain III) of human mu-calpain.

Background/Target Information

The calpains including Calpain 1 (CAPN1), calcium-activated neutral proteases, are nonlysosomal, intracellular cysteine proteases. The mammalian calpains include ubiquitous, stomach-specific, and muscle-specific proteins. The ubiquitous enzymes consist of heterodimers with distinct large, catalytic subunits associated with a common small, regulatory subunit. This gene encodes the large subunit of the ubiquitous enzyme, calpain 1. Several transcript variants encoding two different isoforms have been found for this gene. Diseases associated with CAPN1 include Spastic Paraplegia 76, Autosomal Recessive and Spasticity.

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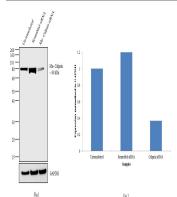
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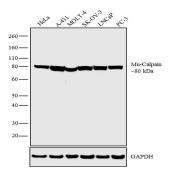


Product Images For Mu-Calpain Monoclonal Antibody (9A4H8D3)



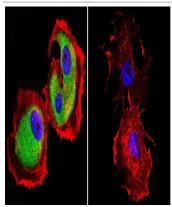
Mu-Calpain Antibody (MA3-940)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. HeLa cells were transfected with Mu-Calpain siRNA and loss of signal was observed in Western Blot using Anti-Mu-Calpain Monoclonal Antibody (Product # MA3-940). {KD}



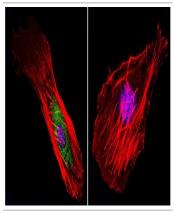
Mu-Calpain Antibody (MA3-940) in WB

Western blot analysis was performed on membrane enriched extracts (30 µg lysate) of HeLa (Lane 1), A-431 (Lane 2), MOLT-4 (Lane 3), SK-OV-3 (Lane 4), LNCaP (Lane 5) and PC-3 (Lane 6). The blot was probed with Mouse Anti-Mu-Calpain Monoclonal Antibody (Product # MA3-940, 2 µg/mL) and detected by chemiluminescence using Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177, 0.25 µg/mL, 1:4000 dilution). An 80 kDa band corresponding to Mu-Calpain was observed across the cell lines tested. Known quantity of protein samples were electrophoresed using Novex® NuPAGE® 4-12 % Bis-Tris gel (Product # NP0321BOX), XCell SureLock™ Electrophoresis System (Product # El0002) and Novex® Sharp Pre-Stained Protein Standard (Product # LC5800). Resolved proteins were then transferred onto a nitrocellulose membrane with iBlot® 2 Dry Blotting System (Product # IB21001). The membrane was probed with the relevant primary and secondary Antibody following blocking with 5 % skimmed milk. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # 32106).



Mu-Calpain Antibody (MA3-940) in ICC/IF

Immunofluorescent analysis of Mu-Calpain in MCF-7 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with a Mu-Calpain monoclonal antibody (Product # MA3-940) at a dilution of 1:20 overnight at 4 C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody (Product # 35503). Mu-Calpain staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification. This image was taken using unpurified ascites antibody.



Mu-Calpain Antibody (MA3-940) in ICC/IF

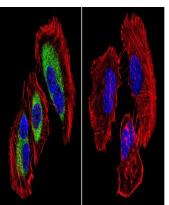
Immunofluorescent analysis of Mu-Calpain in HeLa Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with a Mu-Calpain monoclonal antibody (Product # MA3-940) at a dilution of 1:100 overnight at 4 C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody (Product # 35503). Mu-Calpain staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification. This image was taken using unpurified ascites antibody.

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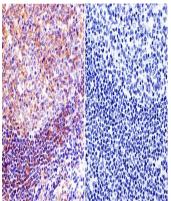




Mu-Calpain Antibody (MA3-940) in ICC/IF

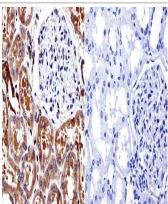
Immunofluorescent analysis of Mu-Calpain in U251 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with a Mu-Calpain monoclonal antibody (Product # MA3-940) at a dilution of 1:20 overnight at 4 C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody (Product # 35503). Mu-Calpain staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification. This image was taken using unpurified ascites antibody.

Mu-Calpain Antibody (MA3-940) in IHC (P)



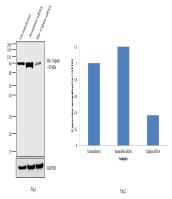
Immunohistochemistry was performed on normal biopsies of deparaffinized human tonsil tissue. To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer, microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:20 with a Mouse Monoclonal Antibody recognizing Mu-Calpain (Product # MA3-940) 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. This image was taken using unpurified ascites antibody.

Mu-Calpain Antibody (MA3-940) in IHC (P)



Immunohistochemistry was performed on normal biopsies of deparaffinized human kidney 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 Mu-Calpain (Product # MA3-940) 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. This image was taken using unpurified ascites antibody.

Mu-Calpain Antibody (MA3-940) in WB



Knockdown of Mu-Calpain was achieved by transfecting HeLa cells with hnRNP K specific validated siRNAs (Silencer® select Product # s491). Western blot analysis (Fig. 1) was performed using whole cell extracts from the Mu-Calpain knockdown cells (lane 3), non-specific scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blots were probed with Anti-Mu-Calpain Monoclonal Antibody (Product # MA3-940, 1 µg/mL) and Goat Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177, 0.25 µg/mL, 1:4000 dilution). Densitometric analysis of this western blot is shown in histogram (Fig. 2). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to Mu-Calpain.

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38 Western Blot Refere	nces
Species / Dilution	Summary
	MA3-940 was used in Western Blot to conclude that deletion of p53 decreases cardiac injury by protecting mitochondria through attenuation of oxidative stress and calpain activation during ischemia-reperfusion.
Mouse / 1:1000	Free radical biology & medicine (2020; 158: 162) "Cardiomyocyte specific deletion of p53 decreases cell injury during ischemia-reperfusion: Role of Mitochondria
	Author(s):Chen Q,Thompson J,Hu Y,Lesnefsky EJ PubMed Article URL:http://dx.doi.org/10.1016/j.freeradbiomed.2020.06.006
	MA3-940 was used in western blot to purify and characterize the calpain and calpastatin from rainbow trout
Fish / Not Cited	Comparative biochemistry and physiology. Part B, Biochemistry & molecular biology (2007; 146: 445) "Purification and characterization of calpain and calpastatin from rainbow trout, Oncorhynchus mykiss." Author(s):Saito M,Li H,Thompson VF,Kunisaki N,Goll DE PubMed Article URL:http://dx.doi.org/10.1016/j.cbpb.2006.10.110
	MA3-940 was used in western blot to investigate the differences in calpain system, desmin degradation, pH values and water holding capacity between muscles of commercial Meishan and Duroc? x ?Landrace? x ?Yorkshire crossbred pigs
Pig / 1:10,000	Animal science journal = Nihon chikusan Gakkaiho (2016; 87: 109) "Differences in calpain system, desmin degradation and water holding capacity between commercial Meishan and DurocxLandracexYorkshire crossbred pork." Author(s):Wang J,Yan XL,Liu R,Fu QQ,Zhou GH,Zhang WG PubMed Article URL:http://dx.doi.org/10.1111/asj.12394
Bovine / Not Cited	MA3-940 was used in western blot to study the biochemical factors governing commercially important beef cut tenderness
	Meat science (2012; 91: 247) "Profile of biochemical traits influencing tenderness of muscles from the beef round." Author(s):Anderson MJ,Lonergan SM,Fedler CA,Prusa KJ,Binning JM,Huff-Lonergan E PubMed Article URL:http://dx.doi.org/10.1016/j.meatsci.2012.01.022
	MA3-940 was used in western blot to study the calpains present in human placenta
Human / Not Cited	Life sciences (2002; 70: 2493) "The calpain system in human placenta." Author(s):Thompson VF,Saldaña S,Cong J,Luedke DM,Goll DE PubMed Article URL:http://dx.doi.org/10.1016/s0024-3205(02)01506-0
	MA3-940 was used in western blot to study the effects on beef quality of electrical input, wrapping, pre rigor temperature and different post rigor chilling rates
Bovine / 1:10000	Meat science (2012; 91: 62) "High pre rigor temperature limits the ageing potential of beef that is not completely overcome by electrical stimulation and muscle restraining." Author(s):Kim YH,Stuart A,Nygaard G,Rosenvold K PubMed Article URL:http://dx.doi.org/10.1016/j.meatsci.2011.12.007
Rat / 1:1000	MA3-940 was used in Western Blotting to show that activation of both cytosolic and mitochondrial calpains impairs mitochondria during cardiac ISC-REP. Mitochondria-localised calpains impair complex I via cleavage for a key subunit.
	American journal of physiology. Cell physiology (2019; 317: C910) "Inhibition of the ubiquitous calpains protects complex I activity and enables improved mitophagy in the heart following ischemia-reperfusion." Author(s):Chen Q,Thompson J,Hu Y,Dean J,Lesnefsky EJ PubMed Article URL:http://dx.doi.org/10.1152/ajpcell.00190.2019
	MA3-940 was used in western blot to study the involvement of protease activity in storage of red swamp crayfish
Arthropod / 1:4000	Journal of agricultural and food chemistry (2008; 56: 8658) "Protease activity in post-mortem red swamp crayfish (Procambarus clarkii) muscle stored in modified atmosphere packaging." Author(s):Chen G,Guttmann RP,Xiong YL,Webster CD,Romaire RP PubMed Article URL:http://dx.doi.org/10.1021/jf8007234

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	MA3-940 was used in western blot to study the effect of 25-hydroxyvitamin D3 and manipulated dietary cation-anion difference on beef quality
Bovine / 1:2000	Journal of animal science (2006; 84: 1481) "Effects of 25-hydroxyvitamin D3 and manipulated dietary cation-anion difference on the tenderness of beef from cull native Korean cows." Author(s):Cho YM,Choi H,Hwang IH,Kim YK,Myung KH PubMed Article URL:http://dx.doi.org/10.2527/2006.8461481x
	MA3-940 was used in western blot to investigate the effect of the high-oxygen modified atmosphere packaging system on oxidation and polymerization
Bovine / Not Cited	Meat science (2010; 85: 759) "High-oxygen modified atmosphere packaging system induces lipid and myoglobin oxidation and protein polymerization." Author(s):Kim YH,Huff-Lonergan E,Sebranek JG,Lonergan SM PubMed Article URL:http://dx.doi.org/10.1016/j.meatsci.2010.04.001
	MA3-940 was used in western blot to study the effect of different aging and freezing protocols on lamb loin tenderness and color stability
Sheep / 1:10,000	Meat science (2013; 95: 412) "Pre rigor processing, ageing and freezing on tenderness and colour stability of lamb loins." Author(s):Kim YH,Luc G,Rosenvold K PubMed Article URL:http://dx.doi.org/10.1016/j.meatsci.2013.05.017
	MA3-940 was used in western blot to study the rate of protein turnover in finisher pigs receiving low residual feed intake
Pig / 1:10,000	Journal of animal science (2013; 91: 4007) "Evidence of decreased muscle protein turnover in gilts selected for low residual feed intake." Author(s):Cruzen SM,Harris AJ,Hollinger K,Punt RM,Grubbs JK,Selsby JT,Dekkers JC,Gabler NK,Lonergan SM,Huff-Lonergan E PubMed Article URL:http://dx.doi.org/10.2527/jas.2013-6413
	MA3-940 was used in western blot to investigate the role of inactivation of mu-calpain for tenderization of beef steaks during oxidative environments
Bovine / 1:10,000	Journal of animal science (2004; 82: 3254) "Oxidative environments decrease tenderization of beef steaks through inactivation of mu-calpain." Author(s):Rowe LJ,Maddock KR,Lonergan SM,Huff-Lonergan E PubMed Article URL:http://dx.doi.org/10.2527/2004.82113254x
Bovine / Not Cited	MA3-940 was used in western blot to evaluate the effect of the postmortem storage time and temperature on calpain activity in bovine muscles
	Journal of animal science (2007; 85: 2670) "Effect of postmortem storage on activity of mu- and m-calpain in five bovine muscles." Author(s):Camou JP,Marchello JA,Thompson VF,Mares SW,Goll DE PubMed Article URL:http://dx.doi.org/10.2527/jas.2007-0164
Bovine / 1:5000	MA3-940 was used in western blot to study the role of small heat shock proteins in toughness of beef with an intermediate ultimate pH
	Meat science (2013; 95: 472) "Small heat shock proteins and toughness in intermediate pHu beef." Author(s):Lomiwes D,Farouk MM,Frost DA,Dobbie PM,Young OA PubMed Article URL:http://dx.doi.org/10.1016/j.meatsci.2013.05.022
Bovine / 1:10,000	MA3-940 was used in western blot to study postmortem muscle proteolysis and fatty acid profiles in beef cattle receiving different amounts of dietary sulfur and the effects of supplemental vitamin C
	Meat science (2014; 96: 956) "Influence of supplemental vitamin C on postmortem protein degradation and fatty acid profiles of the longissimus thoracis of steers fed varying concentrations of dietary sulfur." Author(s):Pogge DJ,Lonergan SM,Hansen SL PubMed Article URL:http://dx.doi.org/10.1016/j.meatsci.2013.08.031
	MA3-940 was used in western blot to investigate the effect of protein denaturation on u-calpain activation
Bovine / 1:10000	Meat science (2010; 86: 883) "Protein denaturing conditions in beef deep semimembranosus muscle results in limited -calpain activation and protein degradation." Author(s):Kim YH,Lonergan SM,Huff-Lonergan E PubMed Article URL:http://dx.doi.org/10.1016/j.meatsci.2010.06.002

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Bovine / 1:40-100	MA3-940 was used in western blot to study the effect of specific calpain antibodies for the regulation of calpain function
	The Journal of biological chemistry (1993; 268: 25740) "Effect of monoclonal antibodies specific for the 28-kDa subunit on catalytic properties of the calpains." Author(s):Cong J,Thompson VF,Goll DE PubMed Article URL:http://www.ncbi.nlm.nih.gov/pubmed/7503986
	MA3-940 was used in Western Blotting to demonstrate that fast freezing then thaw-aging improves water-holding capacity and tenderness by minimizing extracellular ice crystal formation, reducing purge and drip losses, and improving proteolysis in thawed lamb.
Sheep / 1:2500	Animal science journal = Nihon chikusan Gakkaiho (2019; 90: 1060) "Effect of fast freezing then thaw-aging on meat quality attributes of lamb M. longissimus lumborum." Author(s):Balan P,Kim YHB,Stuart AD,Kemp R,Staincliffe M,Craigie C,Farouk MM PubMed Article URL:http://dx.doi.org/10.1111/asj.13216
	MA3-940 was used in western blot to study the effect of pH and ionic strength on mu- and m-calpain activity and the ability of calpastatin to inhibit the activity of mu- or m-calpain
Pig / Not Cited	Journal of animal science (2005; 83: 1370) "Effect of pH and ionic strength on mu- and m-calpain inhibition by calpastatin." Author(s):Maddock KR,Huff-Lonergan E,Rowe LJ,Lonergan SM PubMed Article URL:http://dx.doi.org/10.2527/2005.8361370x
	MA3-940 was used in western blot to investigate the differences in protein modifications between pale, soft, and exudative and red, firm, and nonexudative pork during postmortem aging
Pig / 1:10,000	Journal of animal science (2014; 92: 3745) "Comparison of protein degradation, protein oxidation, and -calpain activation between pale, soft, and exudative and red, firm, and nonexudative pork during postmortem aging." Author(s):Yin Y,Zhang WG,Zhou GH,Guo B PubMed Article URL:http://dx.doi.org/10.2527/jas.2014-7850
	MA3-940 was used in western blot to determine the effect of calcium lactate/phosphate enhancement on the quality of beef round cuts in high-oxygen modified atmosphere
Bovine / 1:5000	Meat science (2015; 101: 63) "Effect of early postmortem enhancement of calcium lactate/phosphate on quality attributes of beef round muscles under different packaging systems." Author(s):Cruzen SM,Kim YH,Lonergan SM,Grubbs JK,Fritchen AN,Huff-Lonergan E PubMed Article URL:http://dx.doi.org/10.1016/j.meatsci.2014.11.004
	MA3-940 was used in western blot to study proteolysis and calpain/calpastatin in three postmortem muscles from young and mature beef cattle and the implications for meat tenderness
Bovine / 1:10,000	Meat science (2014; 96: 854) "Postmortem proteolysis in three muscles from growing and mature beef cattle." Author(s):Cruzen SM,Paulino PV,Lonergan SM,Huff-Lonergan E PubMed Article URL:http://dx.doi.org/10.1016/j.meatsci.2013.09.021
	MA3-940 was used in western blot to investigate the effect of nitric oxide (NO) on calpain activation, protein proteolysis, and oxidation in porcine muscles
Pig / 1:10,000	Journal of agricultural and food chemistry (2014; 62: 5972) "Effect of nitric oxide on -calpain activation, protein proteolysis, and protein oxidation of pork during post-mortem aging." Author(s):Li YP,Liu R,Zhang WG,Fu QQ,Liu N,Zhou GH PubMed Article URL:http://dx.doi.org/10.1021/jf501332d
Bovine / 1:5,000	MA3-940 was used in Western Blotting to indicate that high-fiber diets may negatively impact meat tenderness, and further work is needed to clarify the influence of diet type on parameters of meat tenderness.
	Journal of animal science (2017; 95: 2986) "Influence of feed efficiency classification and growing and finishing diet type on meat tenderness attributes of beef steers." Author(s):Blank CP,Russell J,Lonergan SM,Hansen SL PubMed Article URL:http://dx.doi.org/10.2527/jas.2016.1312
Bovine / 1:1000	MA3-940 was used in Western Blotting to investigate the effects of pulse electric field (PEF) treatment on the tenderization of beef semitendinosus muscle.
	Foods (Basel, Switzerland) (2023; 12:) "Tenderization of Beef <i>Semitendinosus</i> Muscle by Pulsed Electric Field Treatment with a Direct Contact Chamber and Its Impact on Proteolysis and Physicochemical Properties." Author(s):Jeong SH,Jung YM,Kim S,Kim JH,Yeo H,Lee DU PubMed Article URL:http://dx.doi.org/10.3390/foods12030430

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	MA3-940 was used in western blot to study the role of small heat shock proteins in protecting bovine skeletal myofibrils from post-mortem proteolytic degradation
Bovine / 1:5000	Meat science (2014; 97: 548) "The protection of bovine skeletal myofibrils from proteolytic damage post mortem by small heat shock proteins."
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2 Immunocytochemistr	ry References
Species / Dilution	Summary
	MA3-940 was used in immunocytochemistry to show that a calcium-binding ER protein with protective functions agains
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Species / Dilution	Summary
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