Sodium / Calcium Exchanger Antibody (C2C12)

Tested Species Reactivity | Published Species Reactivity
---|---
Human (Hu) | Bovine (Bv)
Mouse (Ms) | Canine (Ca)
Rat (Rt) | Guinea Pig (GP)
Canine (Ca) | Human (Hu)
Rabbit (Rb) | Insect (Ins)
Guinea Pig (GP) | Marsupial (Mr)
 | Mouse (Ms)
 | Porcine (Po)
 | Rabbit (Rb)
 | Rodent (Ro)
 | Rat (Rt)

Tested Applications | Dilution *
---|---
Western Blot (WB) | 1:1,000
Immunofluorescence (IF) | 1:200
Immunohistochemistry (IHC) | 1:20-50
Immunohistochemistry (Frozen) (IHC (F)) | Assay Dependent
Immunohistochemistry (Paraffin) (IHC (P)) | 1/100
Flow Cytometry (FACS) | 1/100
Immunoprecipitation (IP) | Assay dependent

Published Applications | Dilution
---|---
Western Blot (WB) | See publications
Immunocytochemistry (ICC) | See publications
Immunohistochemistry (IHC) | See publications
Immunoprecipitation (IP) | See publications
ELISA (ELISA) | See publications

MA3-926 detects sodium/calcium exchanger from human, canine, rabbit, mouse, guinea pig and rat kidney and cardiac tissues.

MA3-926 has been successfully used in Western blot, immunofluorescence, immunohistochemistry, and immunoprecipitation procedures. By Western blot, this antibody detects a 120 kDa protein representing the sodium/calcium exchanger from guinea pig cardiac extract. The bands seen at 70 kDa and 160 kDa represent a proteolytic fragment and non-reduced exchanger respectively. MA3-926 is not recommended for Western blot procedures of rat tissues. Immunohistochemical staining of the sodium/calcium exchanger in rat heart with MA3-926 results in staining of

**Form Information**

Form: Liquid
Concentration: 0.7 mg/ml
Storage Buffer: ascites diluted in PBS
Preservative: 0.05% sodium azide
Storage Conditions: -20°C, Avoid Freeze/Thaw Cycles

**Details**

Catalog Number: MA3-926
Size: 100 µl
Class: Monoclonal
Type: Antibody
Clone: C2C12
Host / Isotype: Mouse / IgM
Immunogen: Purified canine cardiac sodium/calcium exchanger.

The sodium/calcium exchanger of cardiac sarcolemma rapidly transports calcium during excitation-contraction coupling and is the dominant myocardial calcium efflux mechanism. The sodium/calcium exchanger uses the transmembrane sodium gradient to catalyze countertransport of calcium against its electrochemical gradient in a 3 sodium : 1 calcium exchange. Sodium/calcium exchange activity is present in excitable cells and in non-excitable cells.
the plasma membrane and intense staining of cardiac T-tubular membrane.

The MA3-926 antigen is purified canine cardiac sodium/calcium exchanger. This antibody recognizes an epitope between amino acids 371-525, which is on the intracellular side of the plasma membrane.
Immunofluorescence analysis of Sodium/Calcium Exchanger using Anti-Sodium/Calcium Exchanger Monoclonal Antibody (C2C12) (Product# MA3-926) shows staining in A2058 Cells. Sodium/Calcium Exchanger 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 Sodium/Calcium Exchanger (Product# MA3-926) at a dilution of 1:100 over night at 4 °C, washed with PBS and incubated with a Dylight-488 conjugated secondary antibody (Product# 35503, Goat Anti-Mouse). Images were taken at 60X magnification.

Immunofluorescence analysis of Sodium/Calcium Exchanger using Anti-Sodium/Calcium Exchanger Monoclonal Antibody (C2C12) (Product# MA3-926) shows staining in A549 Cells. Sodium/Calcium Exchanger 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 Sodium/Calcium Exchanger (Product# MA3-926) at a dilution of 1:100 over night at 4 °C, washed with PBS and incubated with a Dylight-488 conjugated secondary antibody (Product# 35503, Goat Anti-Mouse). Images were taken at 60X magnification.

Immunofluorescence analysis of Sodium/Calcium Exchanger using Anti-Sodium/Calcium Exchanger Monoclonal Antibody (C2C12) (Product# MA3-926) shows staining in U251 Cells. Sodium/Calcium Exchanger 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 Sodium/Calcium Exchanger (Product# MA3-926) at a dilution of 1:100 over night at 4 °C, washed with PBS and incubated with a Dylight-488 conjugated secondary antibody (Product# 35503, Goat Anti-Mouse). Images were taken at 60X magnification.

Immunohistochemistry was performed on normal deparaffinized Human heart tissue tissues. 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 Sodium/Calcium Exchanger (MA3-926) 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.

Immunohistochemistry was performed on normal deparaffinized Human kidney tissue tissues. 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 1:50 with a mouse monoclonal antibody recognizing Sodium/Calcium Exchanger (MA3-926) 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.
<table>
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MA3-926 was used in immunocytochemistry and western blot to investigate the expression and localization of sodium/calcium exchanger (NCX1) in human cell lines.

"Molecular determinants of cAMP-mediated regulation of the Na+-Ca2+ exchanger expressed in human cell lines."
Author(s): He LP, Cleemann L, Soldatov NM, Morad M
Number of Citations: 1

MA3-926 was used in western blot to study the functions of hESC-CMs in intracellular calcium handling and the importance of SR in the contraction process.

"Functional properties of human embryonic stem cell-derived cardiomyocytes: intracellular Ca2+ handling and the role of sarcoplasmic reticulum in the contraction."
Number of Citations: 1

MA3-926 was used in western blot to investigate the molecular basis of the aberrant calcium handling during heart failure.

"Cellular and molecular determinants of altered Ca2+ handling in the failing rabbit heart: primary defects in SR Ca2+ uptake and release mechanisms."
Author(s): Sjöström M, Stenström K, Enelning K, Zwiller J, Katz AI, Takemori H, Bertorello AM
Number of Citations: 1

MA3-926 was used in immunocytochemistry and western blot to study the cardiac sodium-calcium exchanger expression in insect cells.

"Expression of the cardiac Na(+)-Ca2+ exchanger in insect cells using a baculovirus vector."
Author(s): Li Z, Smith CD, Smolley JR, Bridge JH, Frank JS, Philipson KD
Number of Citations: 1

MA3-926 was used in western blot to study the role of SK1 in intracellular sodium transport.

"SK1 is part of a cell sodium-sensing network that regulates active sodium transport through a calcium-dependent process."
Author(s): Sjöström M, Stenström K, Enelning K, Zwiller J, Katz AI, Takemori H, Bertorello AM
Number of Citations: 1

MA3-926 was used in western blot to identify the factors related to the rescue of the TOT mouse from the development of a dilated myocardyopathy and premature death by IGF-1.

"Cardiac-specific IGF-1 expression attenuates dilated cardiomyopathy in tropomodulin-overexpressing transgenic mice."
Number of Citations: 1

MA3-926 was used in western blot to study junctin's functions in heart.

"Cardiac remodeling and atrial fibrillation in transgenic mice overexpressing transgenic mice."
Author(s): Hong CS, Cho MC, Kwak YG, Song CH, Lee YH, Lim JS, Kwon YK, Chae SW, Kim DH
Number of Citations: 1

MA3-926 was used in western blot to investigate the effects of overexpression of sodium/calcium exchanger gene on postinfarction myocardial dysfunction.

"Overexpression of Na+Ca2+ exchanger gene attenuates postinfarction myocardial dysfunction."
Number of Citations: 1

MA3-926 was used in immunocytochemistry and western blot to study the expression and localization of sodium/calcium exchanger (NCX1) in human cell lines.

Ms / Not Cited

MA3-926 was used in western blot to investigate the role of S100A1 in the regulation of cardiac function in vivo.


"Transgenic overexpression of the Ca2+-binding protein S100A1 in the heart leads to increased in vivo myocardial contractile performance."


Number of Citations: 1

Ms / 0

MA3-926 was used in western blot to study the mechanism by which triadin 1 overexpression causes a blunted contractile response to beta-adrenergic agonists.


"Transgenic triadin 1 overexpression alters SR Ca2+ handling and leads to a blunted contractile response to beta-adrenergic agonists."


Number of Citations: 6

Ms / Not Cited

MA3-926 was used in immunoprecipitation and western blot to study the role of the sodium potassium ATPase alpha 1 isoform in heart.


"The alpha 1 isoform of Na,K-ATPase regulates cardiac contractility and functionally interacts and co-localizes with the Na/Ca exchanger in heart."

Author(s): Dostanic I, Schultz Jel J, Lorenz JN, Lingrel JB

Number of Citations: 1

Ms / 1:200

MA3-926 was used in western blot to investigate the role of G alpha o on calcium cycling and contractile function in heart.


"Characterization of the phospholemman knockout mouse heart: depressed left ventricular function with increased Na-K-ATPase activity."


Number of Citations: 1

Ms / 1:1000

MA3-926 was used in western blot to investigate the effect of aerobic exercise on calcium signal and redox state of skeletal muscle.


"Aerobic exercise training improves Ca2+ handling and redox status of skeletal muscle in mice."

Author(s): Ferreira JC, Bacurau AV, Bueno CR Jr, Cunha TC, Tanaka LY, Jardim MA, Ramires PR, Brum PC

Number of Citations: 5

Ms / 1:1000

MA3-926 was used in western blot to investigate the effect of aerobic exercise on skeletal muscle function and calcium-related protein expression.


"Aerobic exercise training improves skeletal muscle function and Ca2+ handling-related protein expression in symptomatic hyperactivity-induced heart failure."

Author(s): Bueno CR Jr, Ferreira JC, Pereira MG, Bacurau AV, Brum PC

Number of Citations: 2
MA3-926 was used in immunocytochemistry and western blot to evaluate the caffeine-induced calcium signaling as a differentiation marker of cardiac progenitor cells.


"Caffeine-induced Ca(2+) signaling as an index of cardiac progenitor cells differentiation."

Author(s): Altomare C, Barile L, Marangoni S, Rocchetti M, Alemanni M, Mostacciolo G, Giacomelio A, Messina E, Zaza A

Number of Citations: 3


MA3-926 was used in western blot to investigate the mechanism for the decrease of the TT LTCC current density in failing ventricular myocytes.


"Decrease in the density of t-tubular L-type Ca2+ channel currents in failing ventricular myocytes."


Number of Citations: 1


MA3-926 was used in western blot to study the effects on Ca(2+) metabolism of therapeutic blockade of angiotensin II type 1 receptors.


"Angiotensin receptor blockade improves the net balance of cardiac Ca(2+) handling-related proteins in sympathetic hyperactivity-induced heart failure."

Author(s): Ferreira JC, Moreira JB, Pereira MG, Mattos KC, Coelho MA, Bram PC

Number of Citations: 5


MA3-926 was used in western blot to study the interaction of T-wave alternans and beat-to-beat repolarisation variability in pig heart.


"Microvolt T-wave alternans and beat-to-beat variability of repolarization during early postischemic remodeling in a pig heart."


Number of Citations: 0


MA3-926 was used in western blot to identify the influence of hyperglycemia on ventricular function following myocardial infarction.


"Hyperglycemia can delay left ventricular dysfunction but not autonomic damage after myocardial infarction in rodents."

Author(s): Rodrigues B, Rosa KT, Medeiros A, Schaan BD, Brum PC, De Angelis K, Irigoyen MC

Number of Citations: 8


MA3-926 was used in western blot to investigate the role of sodium/potassium ATPase alpha2 isoform in blood pressure maintenance.


"Knockout of the Na,K-ATPase ?-isoform in the cardiovascular system does not alter basal blood pressure but prevents ACTH-induced hypertension."

Author(s): Rindler TN, Dostanic I, Lasko VM, Nieman ML, Neumann JC, Lorenz JN, Lingrel JB

Number of Citations: 0


MA3-926 was used in western blot to study the phenotype of reduced contractility of the hibernating myocardium.


"Cellular mechanisms of contractile dysfunction in hibernating myocardium."


Number of Citations: 0

This product is for In Vitro experimental use only. Not for resale without express authorization.

Po / 1:1,000

MA3-926 was used in western blot to study the functions of hESC-CMs in intracellular calcium handling and the importance of SR in the contraction process

"Functional properties of human embryonic stem cell-derived cardiomyocytes: intracellular Ca2+ handling and the role of sarcoplasmic reticulum in the contraction."
Number of Citations: 1

Rb / Not Cited

MA3-926 was used in western blot to demonstrate that the overexpression of sodium/calcium exchanger is more susceptible to reactive oxygen species (ROS)-induced injury

Cardiovasc Res. 2003 Nov 1;60(2):404-12.
"Na(+)-Ca(2+) exchanger overexpression predisposes to reactive oxygen species-induced injury."
Number of Citations: 2

Rb / 1:1000

MA3-926 was used in western blot to investigate gene and protein expression of calcium handling proteins after myocardial ischemia in neonatal rabbit heart

"Gene expression of the Na-Ca2+ exchanger, SERCA2a and calsequestrin after myocardial ischemia in the neonatal rabbit heart."
Author(s): Seehase M, Quentin T, Wiludda E, Hellige G, Paul T, Schifflmann H
Number of Citations: 1

Rb / Not Cited

MA3-926 was used in western blot to investigate the molecular basis of the aberrant calcium handling during heart failure.

"Cellular and molecular determinants of altered Ca2+ handling in the failing rabbit heart: primary defects in SR Ca2+ uptake and release mechanisms."
Number of Citations: 1

Rb / Not Cited

MA3-926 was used in western blot to investigate the difference between anterior and posterior myocytes in the left atrium

"Discrepant electrophysiological characteristics and calcium homeostasis of left atrial anterior and posterior myocytes."
Author(s): Suenari K, Chen YC, Kao YH, Cheng CC, Lin YK, Chen YJ, Chen SA
Number of Citations: 1

Rb / 1:1000

MA3-926 was used in immunocytochemistry and western blot to study the effect of estrogen on the regulation of cardiac sodium-calcium exchanger

"Regional genomic regulation of cardiac sodium-calcium exchanger by oestrogen."
Author(s): Chen G, Yang X, Alber S, Shusterman V, Salama G
Number of Citations: 1

Ro / Not Cited

MA3-926 was used in western blot to study mammalian hibernators' inborn cardioprotective functions during hibernation

"Insights into cardioprotection obtained from study of cellular Ca2+ handling in myocardium of true hibernating mammals."
Number of Citations: 1

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Unless otherwise expressly stated on the Product or in the documentation accompanying the Product, the Product is intended for research only and is not to be used for any other purposes, including without limitation, unapproved commercial use, in vivo diagnostic use, or in vitro therapeutic use, or any type of commercial or competitive use, in animal or animal cells.
MA3-926 was used in western blot to investigate the possible relationship between the restoration of the SR calcium-ATPase through adeno viral gene transfer in the aging heart and the normalization of diastolic function in vivo.

"Restoration of diastolic function in senescent rat hearts through adeno viral gene transfer of sarcoplasmic reticulum Ca(2+)-ATPase."

Author(s): Schmidt U, del Monte F, Miyamoto MI, Matsu T, Gwathmey JK, Rosenzweig A, Hajjar RJ
Number of Citations: 10

MA3-926 was used in western blot to study the possible contribution of defects in intracellular calcium signaling cardiomyopathy in streptozotocin (STZ)-induced diabetic rats.

"Defective intracellular Ca(2+) signaling contributes to cardiomyopathy in Type 1 diabetic rats."

Author(s): Choi KM, Zhong Y, Hoit BD, Grupp IL, Hahn H, Dilly KW, Guatimosim S, Lederer WJ, Mattib MA
Number of Citations: 1

MA3-926 was used in western blot to investigate the ability of S100A2 gene transfer to rescue failing myocardium.

"Cardiac adeno viral S100A1 gene delivery rescues failing myocardium."

Number of Citations: 1

MA3-926 was used in western blot to investigate the role of distinct subcellular location of the calcium-binding protein S100A1 in cardiac calcium-handling.

"Distinct subcellular location of the Ca2+-binding protein S100A1 differentially modulates Ca2+-cycling in ventricular rat cardiomyocytes."

Author(s): Fu M, Wu M, Wang JF, Qiao YJ, Wang Z
Number of Citations: 1

MA3-926 was used in western blot to investigate the mechanism of arrhythmic cytotoxicity in heart cells treated with aconitine.

"Disruption of the intracellular Ca2+ homeostasis in the cardiac excitation-contraction coupling is a crucial mechanism of arrhythmic toxicity in aconitine-induced cardiomyocytes."

Author(s): Chen YC, Kao YH, Huang CF, Cheng CC, Chen YJ, Chen SA
Number of Citations: 1

MA3-926 was used in western blot to investigate the effect of heat stress responses on calcium homeostasis and electrophysiological response in atrial myocytes.

"Heat stress responses modulate calcium regulations and electrophysiological characteristics in atrial myocytes."

Author(s): Chen YC, Kao YH, Huang CF, Cheng CC, Chen YJ, Chen SA
Number of Citations: 1

MA3-926 was used in western blot to investigate the mechanism for the protective effect of thiopental against ischemia-reperfusion injury in rats.

"Cardioprotection via modulation of calcium homeostasis by thiopental in hypoxia-reoxygenated neonatal rat cardiomyocytes."

Author(s): Kim HS, Hwang KC, Park WK
Number of Citations: 1
MA3-926 was used in western blot to investigate the relationship between baroreflex dysfunction and heart diastolic function in rodents

"Baroreflex sensitivity impairment is associated with cardiac diastolic dysfunction in rats."
Author(s): Mostarda C, Moraes-Silva IC, Moreira ED, Medeiros A, Piratello A, Consolim-Colombo FM, Caldini EG, Brum PC, Krieger EM, Irgoyen MC
Number of Citations: 1

<table>
<thead>
<tr>
<th>Species / Dilution</th>
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<tr>
<td>GP / 1:10-1:50</td>
<td>MA3-926 was used in western blot and immunocytochemistry to investigate the localization of sodium/calcium exchanger (NCX1) in human cell lines</td>
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<tr>
<td></td>
<td>&quot;Distribution of the Na(+)-Ca2+ exchange protein in mammalian cardiac myocytes: an immunofluorescence and immunocolloidal gold-labeling study.&quot;</td>
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<tr>
<td></td>
<td>Author(s): Frank JS, Mottino G, Reid D, Molday RS, Phillipson KD</td>
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<td>Number of Citations: 32</td>
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<td>Hu / Not Cited</td>
<td>MA3-926 was used in immunocytochemistry and western blot to investigate the expression and localization of sodium/calcium exchanger in cell lines</td>
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<td>J Physiol. 2003 May;1;548(Pt 3):677-89.</td>
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<td>&quot;Molecular determinants of cAMP-mediated regulation of the Na+-Ca2+ exchanger expressed in human cell lines.&quot;</td>
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<td>&quot;Expression of the cardiac Na(+)-Ca2+ exchanger in insect cells using a baculovirus vector.&quot;</td>
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<td>Author(s): Li Z, Smith CD, Smolley JR, Bridge JH, Frank JS, Philipson KD</td>
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<td>Author(s): Altomare C, Barile L, Marangoni S, Rocchetti M, Alemanni M, Mostacciolo G, Giacomello A, Messina E, Zaza A</td>
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<tr>
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<td>Number of Citations: 3</td>
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<tr>
<td>Rb / 1:100</td>
<td>MA3-926 was used in immunocytochemistry to study the densities of the L-type calcium current and proteins related to calcium handling in rabbit sinoatrial node</td>
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<td>&quot;Heterogeneous expression of Ca(2+) handling proteins in rabbit sinoatrial node.&quot;</td>
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<tr>
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<td>Number of Citations: 6</td>
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<td>Rb / 250 ng/ml</td>
<td>MA3-926 was used in immunocytochemistry to investigate the level of sodium/calcium exchanger and caveolin-3 in heart cells during development</td>
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<td>Cell Calcium. 2009 Apr;45(4):369-83.</td>
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<tr>
<td></td>
<td>&quot;Distribution patterns of the Na+−Ca2+ exchanger and caveolin-3 in developing rabbit cardiomyocytes.&quot;</td>
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<td>Author(s): Lin E, Hung VH, Kashihara H, Dan P, Tibbits GF</td>
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### 2 Immunohistochemistry References

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| **Rb / 0**         | MA3-926 was used in immunohistochemistry to investigate the localization of sodium homeostasis-related genes in rabbit heart cells.  
"Colocalization of voltage-gated Na+ channels with the Na+/Ca2+ exchanger in rabbit cardiomyocytes during development."  
Author(s): Gershorne C, Lin E, Kashihara H, Hove-Madsen L, Tibbits GF  
Number of Citations: 1  
| **Rt / 1:100**     | MA3-926 was used in immunohistochemistry to analyze the subcellular distribution of NCX1 and its role in calcium compartmentalization in rat CA1 pyramidal cells.  
"Differential distribution of NCX1 contributes to spine-dendrite compartmentalization in CA1 pyramidal cells."  
Author(s): Lörincz A, Rózsa B, Katona G, Vizi ES, Tamáss G  
Number of Citations: 1  

### 3 Immunoprecipitation References

<table>
<thead>
<tr>
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| **Ca / Not Cited** | MA3-926 was used in immunoprecipitation to investigate the interaction between cardiac sodium/calcium exchanger and ankyrin.  
"The cardiac Na+/Ca2+ exchanger binds to the cytoskeletal protein ankyrin."  
Author(s): Li ZP, Burke EP, Frank JS, Bennett V, Philipson KD  
Number of Citations: 15  
| **Ms / Not Cited** | MA3-926 was used in immunoprecipitation and western blot to study the role of the sodium potassium ATPase alpha 1 isoform in heart.  
"The alpha 1 isoform of Na,K-ATPase regulates cardiac contractility and functionally interacts and co-localizes with the Na/Ca exchanger in heart."  
Author(s): Dostanic I, Schultz Jel J, Lorenz JN, Lingrel JB  
Number of Citations: 1  
| **Rb / 1:100**     | MA3-926 was used in immunoprecipitation to investigate the effect of adiponectin on gluconeogenesis in hepatocytes and its mechanism.  
"Adiponectin represses gluconeogenesis independent of insulin in hepatocytes."  
Author(s): Zhou H, Song X, Briggs M, Violand B, Salsgiver W, Gulve EA, Luo Y  
Number of Citations: 1  

### 1 ELISA Reference

MA3-926 was used in immunoassay and western blot to study the effect of estrogen on the regulation of cardiac sodium-calcium exchanger.

"Regional genomic regulation of cardiac sodium-calcium exchanger by oestrogen."  
Author(s): Chen G, Yang X, Alber S, Shusterman V, Salama G  
Number of Citations: 1  
MA3-926 was used in ELISA and western blot to study the immunoreactive domains of the cardiac sodium-calcium exchanger.

Am J Physiol. 1993 Sep;265(3 Pt 1):C748-56.
"Mapping of the cardiac sodium-calcium exchanger with monoclonal antibodies."
Author(s): Porzig H, Li Z, Nicoll DA, Philipson KD
Number of Citations: 5