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HSC70 Monoclonal Antibody (13D3)

Catalog Number **MA3-014**

Product data sheet

Details	
Size	100 µL
Host/Isotope	Mouse / IgM
Class	Monoclonal
Type	Antibody
Clone	13D3
Immunogen	Mouse spermatogenic cell protein.
Conjugate	Unconjugated
Form	Liquid
Concentration	1 mg/mL
Purification	Affinity chromatography - MBP
Storage buffer	PBS with 1mg/mL BSA
Contains	0.05% sodium azide
Storage Conditions	-20° C, Avoid Freeze/Thaw Cycles

Species Reactivity	
Species reactivity	Cat, Human, Mouse, Non-human primate, Rat
Published species	Rat, Non-human primate, Bovine, Cat, Fish, Human, Mouse, Not Applicable

Tested Applications	Dilution *
Immunohistochemistry (Frozen) (IHC (F))	1:20-1:200
Immunohistochemistry (Paraffin) (IHC (P))	1:200
Immunoprecipitation (IP)	2 µL
Western Blot (WB)	1:1,000-1:4,000
Immunocytochemistry (ICC/IF)	1:250

Published Applications	
Competition Assay (CA)	See 1 publications below
Immunohistochemistry (IHC)	See 1 publications below
Western Blot (WB)	See 13 publications below
Flow Cytometry (Flow)	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

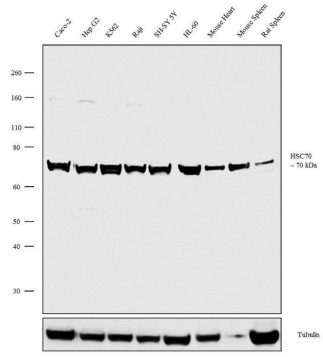
MA3-014 detects heat shock cognate protein 70 kDa (HSC70) from human, feline, primate, rat, and mouse tissues. This antibody displays slight cross-reactivity to HSP70. MA3-014 has been successfully used in Western blot, immunohistochemistry, immunofluorescence, and immunoprecipitation procedures By Western blot, this antibody detects a 70 kDa protein representing HSC70. By 2D gel electrophoresis, MA3-014 was found to bind strongly to HSC70 and faintly to HSP70 both before and after heat shock. Immunohistochemical staining of HSC70 in mouse spermatids/spermatozoa with MA3-014 results in staining restricted to the post acrosomal region in condensing spermatids and to the midpiece in spermatozoa. The MA3-014 antigen is mouse spermatogenic cell protein.

Background/Target Information

The HSP70 family is a set of highly conserved proteins that are induced by a variety of biological stresses, including heat stress, in every organism in which the proteins have been examined. The human HSP70 family members include: HSP70, a protein which is strongly inducible in all organisms but which is also constitutively expressed in primate cells; HSP72, a 72 kDa protein that is induced exclusively under stress conditions; HSC70, or cognate protein, is a 72 kDa, constitutively expressed, protein which is involved in the uncoating of clathrin coated vesicles; GRP78, or BiP, is a glucose regulated 78 kDa protein localized in the endoplasmic reticulum; and p75, or HSP75, a 75 kDa protein that is found within the mitochondria. HSC70 (also known as HSC71, HSC73, HSP73, p72, prp73) is expressed constitutively and is slightly heat-inducible. HSC70 binds to the exposed loop of clathrin light chains to promote uncoating and can also bind the cytoskeleton which may facilitate cytoskeletal rearrangements. HSC70 has been shown to stimulate lysosomal degradation of intracellular proteins and to retard both aggregation and folding of mitochondrial precursor proteins in vitro.

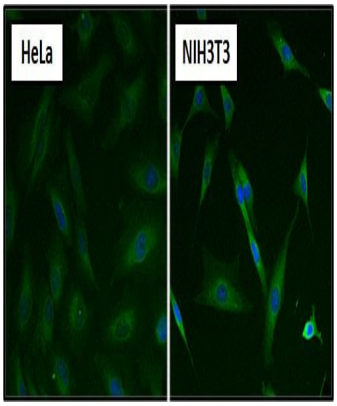
For Research Use Only. Not for use in diagnostic procedures. Not for resale without express authorization.

Product Images For HSC70 Monoclonal Antibody (13D3)



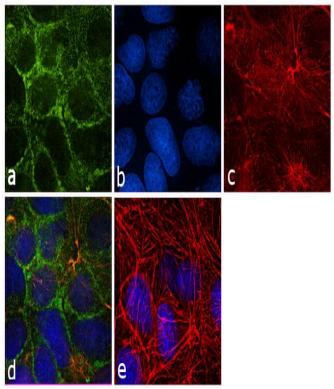
HSC70 Antibody (MA3-014) in WB

Western blot analysis was performed on whole cell extracts (30 µg lysate) of Caco-2 (Lane 1), Hep G2 (Lane 2), K562 (Lane 3), Raji (Lane 4), SH-SY 5Y (Lane 5) HL-60 (Lane 6) tissue extracts of Mouse Brain (Lane 7), Mouse Spleen (Lane 8) and Rat Spleen (Lane 9). The blot was probed with Anti-HSC70 Mouse Monoclonal Antibody (Product # MA3-014, 1:2500 dilution) and detected by chemiluminescence using Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Secondary Antibody, HRP conjugate (Product # A27036, 0.25 µg/mL, 1:4000 dilution). A 70 kDa band corresponding to HSC70 was observed across the cell lines and tissues tested. Known quantity of protein samples were electrophoresed using Novex® NuPAGE® 4-12 % Bis-Tris gel (Product # NP0322BOX), XCell SureLock™ Electrophoresis System (Product # EI0002) 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).



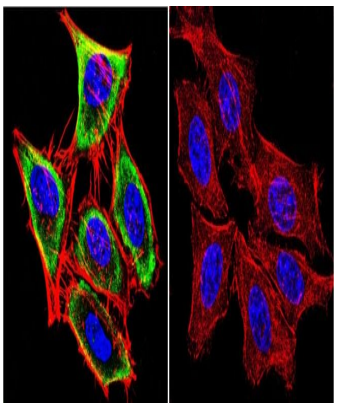
HSC70 Antibody (MA3-014) in ICC/IF

Immunofluorescent analysis of Heat Shock Protein 70 (HSC70) (green) in HeLa and NIH3T3 cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA (Product # 37525) for 15 minutes at room temperature. Cells were probed with a HSC70 monoclonal antibody (Product # MA3-014) at a dilution of 1:50 for at least 1 hour at room temperature, washed with PBS, and incubated with DyLight 488 goat anti-mouse IgG secondary antibody (Product # 35502) at a dilution of 1:400 for 30 minutes at room temperature. Nuclei (blue) were stained with Hoechst 33342 dye (Product # 62249). Images were taken on a Thermo Scientific ArrayScan at 20X magnification.



HSC70 Antibody (MA3-014) in ICC/IF

Immunofluorescence analysis of HSC70/Heat Shock Cognate Protein was performed using 70% confluent log phase Caco-2 cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with HSC70/Heat Shock Cognate Protein Mouse Monoclonal Antibody (Product # MA3-014) at 1:250 dilution in 0.1% BSA and incubated for 3 hours at room temperature and then labeled with Goat anti-Mouse IgG (H+L)/IgM (L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A28175) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing cytoplasmic and membranous localization. Panel e shows the no primary antibody control. The images were captured at 60X magnification.



HSC70 Antibody (MA3-014) in ICC/IF

Immunofluorescent analysis of HSC70 using HSC70 Monoclonal antibody (13D3) (Product # MA3-014) shows staining in HeLa cells. HSC70 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 HSC70 (Product # MA3-014) at a dilution of 1:20-1:200 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.

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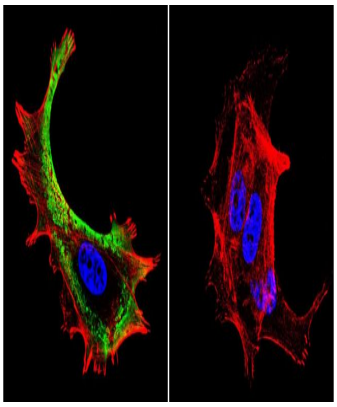
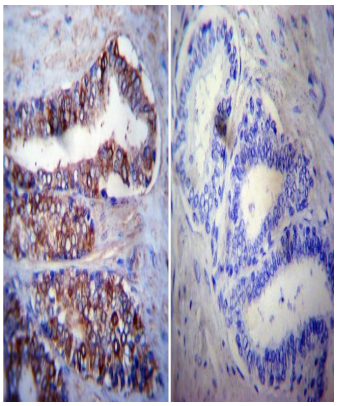


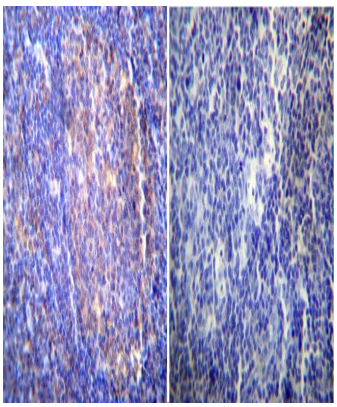
HSC70 Antibody (MA3-014) in ICC/IF

Immunofluorescent analysis of HSC70 using HSC70 Monoclonal antibody (13D3) (Product # MA3-014) shows staining in MCF-7 cells. HSC70 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 HSC70 (Product # MA3-014) at a dilution of 1:20-1:200 °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.



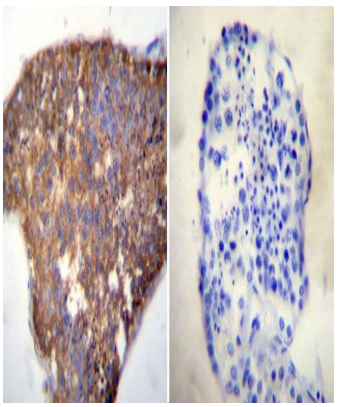
HSC70 Antibody (MA3-014) in IHC (P)

Immunohistochemistry was performed on cancer biopsies of deparaffinized human Prostate carcinoma 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 1:200 with a mouse monoclonal antibody recognizing Heat Shock Cognate Protein 70 (Product # MA3-014) 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.



HSC70 Antibody (MA3-014) in IHC (P)

Immunohistochemistry was performed on normal 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 Heat Shock Cognate Protein 70 (Product # MA3-014) 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.



HSC70 Antibody (MA3-014) in IHC (P)

Immunohistochemistry was performed on normal deparaffinized human 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 Heat Shock Cognate Protein 70 (Product # MA3-014) 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.

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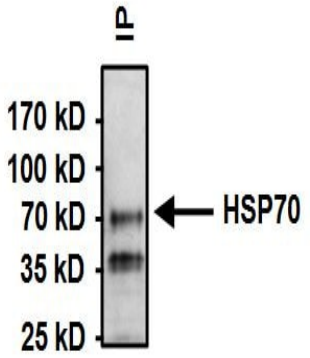
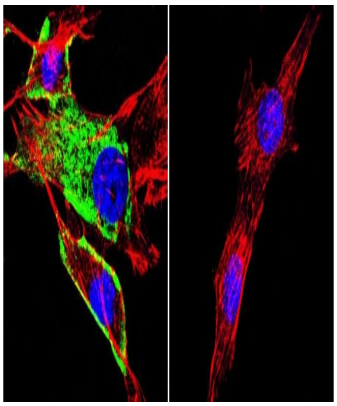


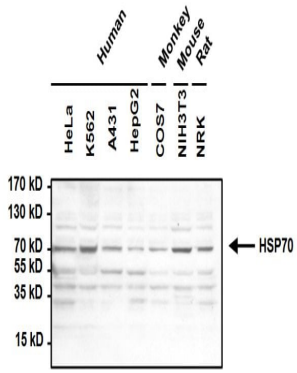
HSC70 Antibody (MA3-014) in IP

Immunoprecipitation of HSC70 was performed on HeLa cells. Antigen-antibody complexes were formed by incubating 500 µg whole cell lysate with 2 µL of HSC70 monoclonal antibody (Product # MA3-014) overnight on a rocking platform at 4°C. The immune complexes were captured on 50 µL Protein A/G Plus Agarose (Product # 20421), washed extensively, and eluted with Lane Marker Reducing Sample Buffer (Product # 39000). Samples were resolved on a 4-20% Tris-HCl polyacrylamide gel, transferred to a PVDF membrane, and blocked with 5% BSA/TBST for at least 1 hour. The membrane was probed with a HSC70 monoclonal antibody (Product # MA3-014) at a dilution of 1: 1000 overnight rotating at 4°C, washed in TBST, and probed with goat anti-mouse IgM-HRP secondary antibody (Product # 31440) for at least 1 hour. Chemiluminescent detection was performed using SuperSignal West Dura (Product # 34075).



HSC70 Antibody (MA3-014) in ICC/IF

Immunofluorescent analysis of HSC70 using Monoclonal antibody (13D3) (Product # MA3-014) shows staining in NIH-3T3 cells. HSC70 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 HSC70 (Product # MA3-014) at a dilution of 1:20-1:200 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.



HSC70 Antibody (MA3-014) in WB

Western blot analysis of HSC70 was performed by loading 50 µg of the indicated whole cell lysates and 15 µL of PageRuler Prestained Protein Ladder (Product # 26616) onto a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to a PVDF membrane and blocked with 5% BSA/TBST for at least 1 hour. The membrane was probed with a HSC70 monoclonal antibody (Product # MA3-014) at a dilution of 1:1000 overnight at 4°C on a rocking platform, washed in TBS-0.1%Tween 20, and probed with a goat anti-mouse IgM-HRP secondary antibody (Product # 31440) at a dilution of 1:20,000 for at least 1 hour. Chemiluminescent detection was performed using SuperSignal West Pico (Product # 34080).

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PubMed References For HSC70 Monoclonal Antibody (13D3)

1 Competition Assay References

Species / Dilution	Summary
/ Not Cited Human / Not Cited	MA3-014 was used in Competition Assay to provide data which denotes the importance of careful evaluation of reagents used to identify receptor use in invertebrate cells. Frontiers in cell and developmental biology (2023; 11:) **"Chikungunya virus entry and infectivity is primarily facilitated through cell line dependent attachment factors in mammalian and mosquito cells."** Author(s):Reyes Ballista JM,Miazgowicz KL,Acciani MD,Jimenez AR,Belloli RS,Havranek KE,Brindley MA PubMed Article URL:http://dx.doi.org/10.3389/fcell.2023.1085913

1 Immunohistochemistry References

Species / Dilution	Summary
Rat / 1:200	MA3-014 was used in immunohistochemistry and western blot to study the expression of small heat shock proteins in the retina of diabetic rats Investigative ophthalmology & visual science (2013; 54: 7674) **"Response of small heat shock proteins in diabetic rat retina."** Author(s):Reddy VS,Raghu G,Reddy SS,Pasupulati AK,Suryanarayana P,Reddy GB PubMed Article URL:http://dx.doi.org/10.1167/iovs.13-12715

13 Western Blot References

Species / Dilution	Summary
Human / 1:1000	MA3-014 was used in western blot to characterize the proteomic content of lymphoma cell-derived exosomes. European journal of medical research (2015; 20:) **"Proteomic analysis of exosomes derived from human lymphoma cells."** Author(s):Yao Y,Wei W,Sun J,Chen L,Deng X,Ma L,Hao S PubMed Article URL:http://dx.doi.org/10.1186/s40001-014-0082-4
Human / 1:1000	MA3-014 was used in western blot to investigate the changes of HSP27 and HSP70 expression induced by eccentric exercise in humans Acta physiologica Scandinavica (2002; 174: 47) **"The repeated bout effect and heat shock proteins: intramuscular HSP27 and HSP70 expression following two bouts of eccentric exercise in humans."** Author(s):Thompson HS,Clarkson PM,Scordilis SP PubMed Article URL:http://dx.doi.org/10.1046/j.1365-201x.2002.00922.x
Mouse / Not Cited	MA3014 was used in western blot to elucidate the contribution of the Sac phosphatase domain of Synaptojanin 1 to neurological symptoms Neuron (2017; 93: 882) **"Parkinson Sac Domain Mutation in Synaptojanin 1 Impairs Clathrin Uncoating at Synapses and Triggers Dystrophic Changes in Dopaminergic Axons."** Author(s):Cao M,Wu Y,Ashrafi G,McCartney AJ,Wheeler H,Bushong EA,Boassa D,Ellisman MH,Ryan TA,De Camilli P PubMed Article URL:http://dx.doi.org/10.1016/j.neuron.2017.01.019
Fish / Not Cited	MA3-014 was used in western blot to characterize a new Nm23/nucleoside diphosphate kinase family member The Journal of biological chemistry (1997; 272: 2607) **"A 16-kDa protein functions as a new regulatory protein for Hsc70 molecular chaperone and is identified as a member of the Nm23/nucleoside diphosphate kinase family."** Author(s):Leung SM,Hightower LE PubMed Article URL:http://dx.doi.org/10.1074/jbc.272.5.2607
Not Applicable / 1:1000	MA3-014 was used in western blot to study the interaction of components of the cytoskeleton with two new isoforms of the human hepatoma-derived growth factor Biological chemistry (2016; 397: 417) **"Two new isoforms of the human hepatoma-derived growth factor interact with components of the cytoskeleton."** Author(s):Nüße J,Mirastschijski U,Waespy M,Oetjen J,Brandes N,Rebello O,Paroni F,Kelm S,Dietz F PubMed Article URL:http://dx.doi.org/10.1515/hsz-2015-0273
Mouse / Not Cited	MA3-014 was used in Western Blot to find that the NSPC chaperone network robustly maintains misfolded protein solubility and stress resilience through high levels of the ATP-dependent chaperonin TRiC/CCT. Molecular cell (2020; 78: 329) **"Differentiation Drives Widespread Rewiring of the Neural Stem Cell Chaperone Network."** Author(s):Vonk WIM,Rainbolt TK,Dolan PT,Webb AE,Brunet A,Frydman J PubMed Article URL:http://dx.doi.org/10.1016/j.molcel.2020.03.009

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Human / 1:1,000	MA3-014 was used in western blot to characterize gene expression in the primate retinas during aging. Investigative ophthalmology & visual science (2000; 41: 2857) **"Heat shock cognate-70 gene expression declines during normal aging of the primate retina."** Author(s):Bernstein SL,Liu AM,Hansen BC,Somiari RI PubMed Article URL:http://www.ncbi.nlm.nih.gov/pubmed/10967038
Rat / 1:500	MA3-014 was used in western blot to examine the expression of sHsp under chronic hyperglycemic conditions in rat heart Archives of biochemistry and biophysics (2014; 558: 1) **"Expression and induction of small heat shock proteins in rat heart under chronic hyperglycemic conditions."** Author(s):Reddy VS,Kumar ChU,Raghu G,Reddy GB PubMed Article URL:http://dx.doi.org/10.1016/j.abb.2014.06.008
Mouse / Not Cited	MA3-014 was used in western blot to investigate the distribution of hsp70-like protein in mouse germ cells Molecular and cellular biology (1988; 8: 828) **"A novel hsp70-like protein (P70) is present in mouse spermatogenic cells."** Author(s):Allen RL,O'Brien DA,Eddy EM PubMed Article URL:http://dx.doi.org/10.1128/mcb.8.2.828-832.1988
Cat / 1:5,000	MA3-014 was used in western blot to investigate the consequences of inhibiting the upregulation of endogenous HSP 72 in cardiac myocytes in response to hypoxic stress Circulation (1997; 95: 1523) **"Blocking the endogenous increase in HSP 72 increases susceptibility to hypoxia and reoxygenation in isolated adult feline cardiocytes."** Author(s):Nakano M,Mann DL,Knowlton AA PubMed Article URL:http://dx.doi.org/10.1161/01.cir.95.6.1523
Mouse / Not Cited	MA3-014 was used in western blot to investigate the association between HSF-1 and HSC 70 protein in the cytoplasm of NIH-3T3 cells. Biochemical and biophysical research communications (1995; 213: 1) **"Heat shock factor-1 and the heat shock cognate 70 protein associate in high molecular weight complexes in the cytoplasm of NIH-3T3 cells."** Author(s):Nunes SL,Calderwood SK PubMed Article URL:http://dx.doi.org/10.1006/bbrc.1995.2090
Bovine / 1:500	MA3-014 was used in western blot to investigate the functional properties of hsc73 The Journal of biological chemistry (1992; 267: 9202) **"Protein and peptide binding and stimulation of in vitro lysosomal proteolysis by the 73-kDa heat shock cognate protein."** Author(s):Terlecky SR,Chiang HL,Olson TS,Dice JF PubMed Article URL:http://www.ncbi.nlm.nih.gov/pubmed/1577755
Human / Not Cited	MA3-014 was used in western blot to conduct the proteomic profiling of exosomes secreted by human mesothelioma cells. The American journal of pathology (2004; 164: 1807) **"Proteomic analysis of exosomes secreted by human mesothelioma cells."** Author(s):Hegmans JP,Bard MP,Hemmes A,Luider TM,Kleijmeer MJ,Prins JB,Zitvogel L,Burgers SA,Hoogsteden HC,Lambrecht BN PubMed Article URL:http://dx.doi.org/10.1016/S0002-9440(10)63739-X

1 Flow Cytometry References

Species / Dilution	Summary
Non-human primate / Not Cited	MA3-014 was used in flow cytometry to study the role of integrin subunits alpha2 and beta3 in rotavirus cell entry using RNA silencing Virus research (2009; 145: 251) **"Dissecting the role of integrin subunits alpha 2 and beta 3 in rotavirus cell entry by RNA silencing."** Author(s):Isa P,Sánchez-Alemán MA,López S,Arias CF PubMed Article URL:http://dx.doi.org/10.1016/j.virusres.2009.07.013

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