

Aggrecan Monoclonal Antibody (BC-3)

Product Details	
Size	100 µg
Species Reactivity	Bovine, Dog, Horse, Cat, Guinea pig, Human, Sheep, Pig, Rabbit, Rat
Published Species	Bacteria, Zebrafish, Mouse, Human
Host/Isotope	Mouse / IgG1, kappa
Class	Monoclonal
Type	Antibody
Clone	BC-3
Conjugate	Unconjugated
Immunogen	Synthetic peptide corresponding to human aggrecan ARGSV neoepitope.
Form	Liquid
Concentration	1 mg/mL
Purification	Protein A
Storage buffer	PBS with 1mg/mL BSA, 30% glycerol
Contains	0.05% sodium azide
Storage Conditions	-20°C
RRID	AB_568440

Applications	Tested	Dilution	Published
Immunofluorescence (IF)	✓	2 µg/mL	2 Publications
Western Blot (WB)	✓	1:500 - 1:1000	3 Publications
Immunohistochemistry (IHC)	-	1:30	4 Publications
Immunohistochemistry (Frozen) (IHC (F))	✓	1:10 - 1:2000	3 Publications
Immunohistochemistry (Paraffin) (IHC (P))	✓	1:10 - 1:2000	2 Publications
Immunocytochemistry (ICC)	✓	2 µg/mL	1 Publication
ELISA (ELISA)	✓	1:1000 - 1:5000	

Product Specific Information

Purified recombinant Aggrecan Interglobular Domain (IGD) protein (Amino acids T331-G458 with C-term His-tag, expressed in E. coli), was used to validate this antibody. The recombinant protein contains cleavage sites for aggrecanases (E373-A374 in aggrecan) and matrix metalloproteinases (N341-F342 in aggrecan). The recombinant protein was cleaved in vitro using recombinant Aggrecanase. Degradation of IGD and accumulation of cleaved fragments was visualized via MEMCode reversible protein staining, followed by Western Blot using the BC-3 antibody for the specific detection of the aggrecan neoepitope. The antibody will also recognize full length IGD, as shown by ELISA, however with significantly lower affinity.

MA3-16888 has successfully been used in ELISA, immunohistochemistry (frozen and FFPE) and Western blot applications. This antibody may be used to immunostain formalin-fixed, paraffin-embedded sections, as well as alcohol-fixed frozen sections and un-

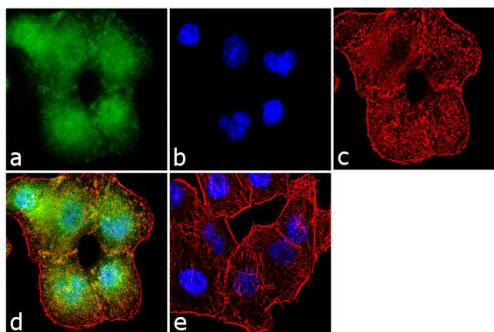
fixed snap frozen sections. Samples must be deglycosylated using 0.01 Units Chondroitinase ABC, 0.01 Units Keratanase and 0.0001 Units Keratanase II per 10 µg S-GAG of non-deglycosylated aggrecan for optimal epitope recognition.

MA3-16888 detects aggrecan in human, bovine, feline, canine, guinea pig, equine, porcine, rabbit, rat, and sheep samples. Does not react with mouse samples.

Product Images For Aggrecan Monoclonal Antibody (BC-3)

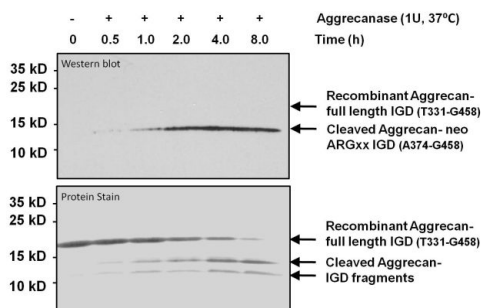
Aggrecan Antibody (MA3-16888) in IF

Immunofluorescence analysis of Aggrecan was performed using 70% confluent log phase A549 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 Aggrecan (BC-3) Mouse Monoclonal Antibody (Product # MA3-16888) at 2 µg/mL in 0.1% BSA and incubated for 3 hours at room temperature and then labeled with Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conj µgate (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 Alexa Fluor® 555 Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing localization in the extracellular matrix. Panel e shows the no primary antibody control. The images were captured at 60X magnification.



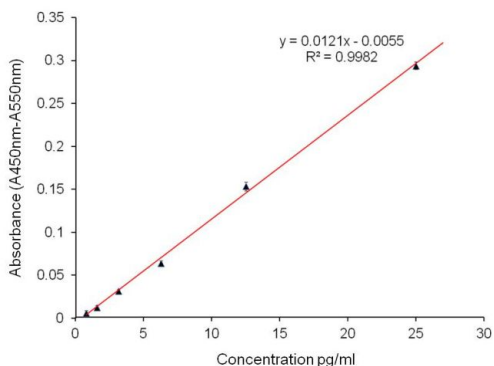
Aggrecan Antibody (MA3-16888) in WB

Western blot analysis of Aggrecan neopeptide fragments, generated as a result of Aggrecanase proteolytic activity, was performed using cleaved and uncleaved recombinant Interglobular domain (IGD) of Aggrecan (Product # RP-77527), followed by detection with an Aggrecan monoclonal antibody (Product # MA3-16888). IGD (5 µg) was incubated for the indicated times with or without 1U recombinant Aggrecanase. IGD samples and 10 µL of PageRuler Prestained Protein Ladder (Product # 26616) were loaded onto a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to a PVDF membrane and full length IGD, as well as cleaved Aggrecan-IGD fragments were visualized (bottom panel) using a Reversible Protein Stain Kit for PVDF Membranes (Product # 24585). For specific neo-epitope fragment detection (top panel), the membrane was incubated in Destain solution (included in the kit), blocked with 5% BSA/TBST for 1 hour, and probed with an Aggrecan monoclonal antibody (Product # MA3-16888) at a dilution of 1:1000 overnight at 4°C on a rocking platform. The membrane was washed in TBS-0.1% Tween-20, and probed with a goat anti-mouse IgG-HRP secondary antibody (Product # 31430) at a dilution of 1:20,000 for 1 hour. Chemiluminescent detection was performed using SuperSignal West Pico (Product # 34080).



Aggrecan Antibody (MA3-16888) in ELISA

ELISA analysis of Aggrecan was performed by coating Amine-binding Maleic Anhydride Activated Plates (Product # 15110) overnight with 100 µL of 2 µg/mL Aggrecan Interglobular Domain recombinant protein (Product # RP-77527). Plates were blocked for 1 hour at room temperature with StartingBlock Blocking Buffer (Product # 37538) and incubated with 100 µL per well of an Aggrecan monoclonal antibody (Product # MA3-16888) in duplicate at 25, 12.5, 6.25, 3.125, 1.563, 0.78 µg /100 µL with serial dilutions of for 2 hours at room temperature. The plate was washed and incubated with 100 µL per well of goat anti-mouse IgG-HRP secondary antibody (Product # 31430) in all test wells at 1:30,000 for 1 hour at room temperature. Detection was performed using 1-Step Ultra TMB - ELISA Substrate (Product # 34028) for 30 minutes at room temperature in the dark. The plate was then stopped with 0.16M sulfuric acid. Absorbance values were read on a spectrophotometer at 450-550 nm.



15 References

Immunofluorescence (2)

Molecular medicine reports

Andrographolide mitigates IL1induced human nucleus pulposus cells degeneration through the TLR4/MyD88/NFB signaling pathway.

"Published figure using Aggrecan monoclonal antibody (Product # MA3-16888) in Immunofluorescence"

Authors: Zhang L,Chen Q,Wang H,Yang J,Sheng S

Species

Not Applicable

Dilution

Not Cited

Year

2018

PloS one

Cx43-Dependent Skeletal Phenotypes Are Mediated by Interactions between the Hapln1a-ECM and Sema3d during Fin Regeneration.

"Published figure using Aggrecan monoclonal antibody (Product # MA3-16888) in Immunofluorescence"

Authors: Govindan J,Tun KM,Iovine MK

Species

Not Applicable

Dilution

Not Cited

Year

2016

Western Blot (3)

Molecular medicine reports

Andrographolide mitigates IL1induced human nucleus pulposus cells degeneration through the TLR4/MyD88/NFB signaling pathway.

"Published figure using Aggrecan monoclonal antibody (Product # MA3-16888) in Immunofluorescence"

Authors: Zhang L,Chen Q,Wang H,Yang J,Sheng S

Species

Not Applicable

Dilution

Not Cited

Year

2018

Arthritis research and therapy

Proteomic profiling and functional characterization of early and late shoulder osteoarthritis.

"Published figure using Aggrecan monoclonal antibody (Product # MA3-16888) in Western Blot"

Authors: Wanner J,Subbaiah R,Skomorovska-Prokvolit Y,Shishani Y,Boilard E,Mohan S,Gillespie R,Miyagi M,Gobezie R

Species

Human
Not Applicable

Dilution

Not Cited
Not Cited

Year

2013

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More applications with references on thermofisher.com

IHC (4)

IHC (F) (3)

IHC (P) (2)

ICC (1)

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