

FOXO1 Monoclonal Antibody (3B6)

Catalog NumberMA5-17078

Product data sheet

Details		Species Reactivity	
Size	100 µg	Species reactivity	Human, Mouse
Host/Isotope	Mouse / IgG1	Published species	Mouse, Human
Class	Monoclonal	Tested Applications	
Type	Antibody	ChIP assay (ChIP)	Dilution *Assay-dependent
Clone	3B6	Immunohistochemistry (Paraffin) (IHC (P))	1:200-1:1,000
Immunogen	Purified recombinant fragment of human FOXO1 expressed in E. Coli.	Western Blot (WB)	1:500-1:1,000
Conjugate	Unconjugated	Immunocytochemistry (ICC/IF)	1:100-1:200
Form	Liquid	Published Applications	
Concentration	1 mg/mL	Western Blot (WB)	See 1 publications below
Purification	Protein A	Immunocytochemistry (ICC/IF)	See 1 publications below
Storage buffer	PBS	* 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.	
Contains	0.05% sodium azide		
Storage Conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.		

Product specific information

MA5-17078 targets FOXO1 in ChIP, IHC, IF, and WB applications and shows reactivity with Human and Mouse samples. The MA5-17078 immunogen is purified recombinant fragment of human FOXO1 expressed in E. Coli. MA5-17078 detects FOXO1 which has a predicted molecular weight of approximately 69.7kDa.

Background/Target Information

FOXO1 (FKHR, ForkHead Box 01) is a member in a subfamily of the forkhead homeotic gene family of transcription factors. Recent experiments have shown that FOXO1 can act as either a coactivator or a corepressor of nuclear receptor activity that is mediated through the LXXLL motif found in the carboxyl terminal region of the FKHR protein. Association of FOXO1 with PAX3 has been implicated in alveolar rhabdomyosarcoma. Recent studies link the anti-tumor activity of FOXO1, the process of autophagy and myogenic growth and differentiation. FOXO1 is the main target of insulin signaling that regulated the metabolic homeostasis in response to oxidative stress. FOXO1 binds to the insulin response element (IRE) with consensus sequence 5'-TT[G/A]TTTTG-3' and the related Daf-16 family binding element (DBE) with consensus sequence 5'-TT[G/A]TTTAC-3'. FOXO1 is a regulator of redox balance, osteoblast numbers and controls bone mass. Further, FOXO1 orchestrates the endocrine function of the skeleton in regulating glucose metabolism, and suppresses the transcriptional activity of RUNX2, an upstream activator of osteocalcin/BGLAP. In hepatocytes, FOXO1 promotes gluconeogenesis by acting together with PPARGC1A to activate the expression of genes such as IGFBP1, G6PC and PPCK1. FOXO1 is an important regulator of cell death acting downstream of CDK1, PKB/AKT1 and SKT4/MST1.

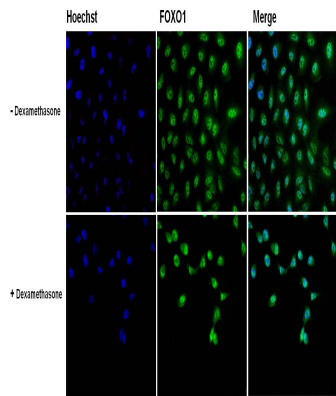
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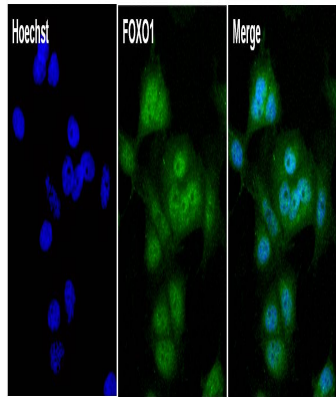
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Product Images For FOXO1 Monoclonal Antibody (3B6)



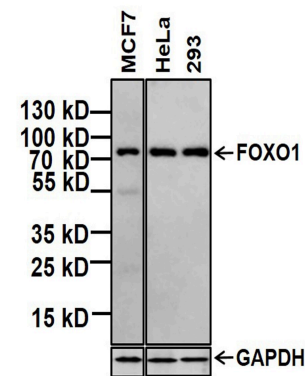
FOXO1 Antibody (MA5-17078) in ICC/IF

Immunofluorescent analysis of FOXO1 (green) in untreated and 1uM 20 hours Dexamethasone treated A549 cells. The cells were fixed with 4% paraformaldehyde for 15 minutes at -20c, permeabilized with 0.1% Triton X-100 for 15 minutes, and blocked with 3% BSA for 30 minutes at room temperature. Cells were stained with a FOXO1 mouse monoclonal antibody (Product # MA5-17078) at a concentration of 5 µg/mL in blocking buffer for 1 hour at room temperature, and then incubated with a Goat anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor Plus 488 conjugate (Product # A32731) at a dilution of 1:500 for at least 30 minutes at a room temperature in the dark (green). Nuclei (blue) were stained with Hoechst 33342 (Product # 62249). Images were taken on a Thermo Scientific ToxInsight Instrument at 20X magnification.



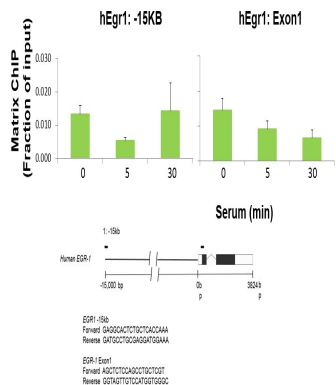
FOXO1 Antibody (MA5-17078) in ICC/IF

Immunofluorescent analysis of FOXO1 (green) in MCF7 cells. The cells were fixed with 4% paraformaldehyde for 15 minutes at -20c, permeabilized with 0.1% Triton X-100 for 15 minutes, and blocked with 3% BSA for 30 minutes at room temperature. Cells were stained with a FOXO1 mouse monoclonal antibody (Product # MA5-17078) at a concentration of 5 µg/mL in blocking buffer for 1 hour at room temperature, and then incubated with a Goat anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor Plus 488 conjugate (Product # A32731) at a dilution of 1:500 for at least 30 minutes at a room temperature in the dark (green). Nuclei (blue) were stained with Hoechst 33342 (Product # 62249). Images were taken on a Thermo Scientific ToxInsight Instrument at 20X magnification.



FOXO1 Antibody (MA5-17078) in WB

Western blot analysis of FOXO1 was performed by loading 20 µg of MCF7 whole cell lysate and 7 µL of PageRuler Plus Prestained Protein Ladder (Product # 26619) per well onto a 4-20% Tris-Glycine polyacrylamide gel (Product # WT4202BX10). Proteins were transferred to a nitrocellulose membrane using the G2 Blotter (Product # 62288), and blocked with 5% BSA in TBST (Product # 37520) for 1 hour at room temperature. FOXO1 was detected at ~72 kDa using a FOXO1 mouse monoclonal antibody (Product # MA5-17078) at a dilution of 1:500 in blocking buffer overnight at 4°C on a rocking platform, followed by a Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177) at a dilution of 1:1000 for at least 30 minutes at room temperature. dy, HRP conjugate (Product # A28177) at a dilution of 1:2000 for at least 30 minutes at room temperature. Chemiluminescent detection was performed using SuperSignal West Pico (Product # 34078).



FOXO1 Antibody (MA5-17078) in ChIP

Multiplex microplate Matrix ChIP has been described in detail (<http://www.ncbi.nlm.nih.gov/pubmed/25959381>). Briefly HTC116 cells were starved followed by addition of serum and samples of cells were cross-linked with formaldehyde after the time points indicated on the x-axis (0, 5 and 30 min). Chromatin was sheared using a Bioruptor and ChIP assays were performed using protein A-coated 96-well polypropylene microplates with 1uL/100uL well volume of FOXO1 monoclonal antibody (Product # MA5-17078). Quantitative real-time PCRs were performed in quadruplicate using 1 to 2uL of DNA with primers to -15kb downstream of Egr1 and exon 1 of Egr1. PCR calibration curves were generated for each primer pair from a dilution series of total human genomic DNA. The PCR primer efficiency curve was fit to cycle threshold (Ct) versus log [genomic DNA concentration] by using an r2 best fit. DNA concentration values for each ChIP and input DNA sample were calculated from their respective average Ct values. Final results are expressed as fraction of input DNA. Schematic representations of Egr1 loci is shown where boxes represent exons (black boxes = translated regions, white boxes = untranslated regions), the zigzag line represents an intron, and the straight line represents upstream sequence. Regions amplified by the primers are represented by black bars. Data courtesy of Dr. Karol Bomsztyk's laboratory.

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PubMed References For FOXO1 Monoclonal Antibody (3B6)

1 Western Blot References

Species / Dilution	Summary
	MA5-17078 was used in Western blot to conclude that mesangial cell damage and extracellular remodelling caused by hyperhomocysteinemia is regulated by the Akt/FOXO1 pathway.
Mouse / Not Cited	Cellular signalling (2019; 61: 66) "Hydrogen sulphide mitigates homocysteine-induced apoptosis and matrix remodelling in mesangial cells through Akt/FOXO1 signalling cascade." Author(s):Majumder S,Ren L,Pushpakumar S,Sen U PubMed Article URL: http://dx.doi.org/10.1016/j.cellsig.2019.05.003

1 Immunocytochemistry References

Species / Dilution	Summary
	MA5-17078 was used in Immunocytochemistry-immunoflourescence to conclude that our culture system enables a reductionist investigation of regulation of human NSC plasticity for the identification of potential therapeutic targets for intervention in AD.
Human / 1:100	Developmental cell (2018; 46: 85) "3D Culture Method for Alzheimer's Disease Modeling Reveals Interleukin-4 Rescues A42-Induced Loss of Human Neural Stem Cell Plasticity." Author(s):Papadimitriou C,Celikkaya H,Cosacak MI,Mashkaryan V,Bray L,Bhattarai P,Brandt K,Hollak H,Chen X,He S,Antos CL,Lin W,Thomas AK,Dahl A,Kurth T,Friedrichs J,Zhang Y,Freudenberg U,Werner C,Kizil C PubMed Article URL: http://dx.doi.org/10.1016/j.devcel.2018.06.005