

See 1 publications below





EZH2 Monoclonal Antibody (144CT2.1.1.5)

Catalog Number MA5-18108 Product data sheet

Species Reactivity

Published Applications
Immunocytochemistry (ICC/IF)

Details	
Size	400 μL
Host/Isotope	Mouse / IgG1, kappa
Class	Monoclonal
Туре	Antibody
Clone	144CT2.1.1.5
Immunogen	EZH2 recombinant protein
Conjugate	Unconjugated
Form	Liquid
Concentration	0.5 mg/mL
Purification	Protein G
Storage buffer	PBS
Contains	0.09% sodium azide
Storage Conditions	-20° C, Avoid Freeze/Thaw Cycles

Species reactivity	Human
Published species	Human, Not Applicable
Tested Applications	Dilution *
ChIP assay (ChIP)	4 μL/10^6 cells
Immunohistochemistry (Paraffin) (IHC (P))	1:25
Western Blot (WB)	1:2,000
Immunocytochemistry (ICC/IF)	1:25

^{*} 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

This antibody is predicted to react with non-human primate based on sequence homology.

Background/Target Information

EZH2 (Histone-lysine N-methyltransferase) is a member of the Polycomb-group (PcG) family. PcG family members form multimeric protein complexes, which are involved in maintaining the transcriptional repressive state of genes over successive cell generations. This protein associates with the embryonic ectoderm development protein, the VAV1 oncoprotein, and the X-linked nuclear protein. EZH2 may play a role in the hematopoietic and central nervous systems. Mutations affecting EZH2 can cause Weaver syndrome (WVS).

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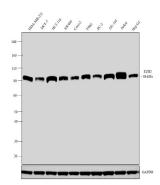
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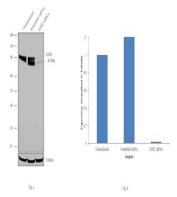


Product Images For EZH2 Monoclonal Antibody (144CT2.1.1.5)



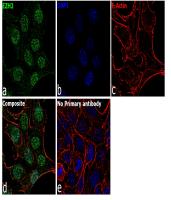
EZH2 Antibody (MA5-18108) in WB

Western blot analysis was performed on whole cell extracts (30 μg lysate) of MDA-MB-231 (Lane 1), MCF7 (Lane 2), HCT 116 (Lane 3), SW480 (Lane 4), Caco-2 (Lane 5), T98G (Lane 6), PC-3 (Lane 7), DU 145 (Lane 8), Jurkat (Lane9) and Hep G2 (Lane 10). The blot was probed with Anti-EZH2 Monoclonal Antibody (144CT2.1.1.5) (Product # MA5-18108, 1:2000 dilution) and detected by chemiluminescence using Goat anti Mouse IgG (H+L) SuperclonalTM Secondary Antibody, HRP conjugate (Product # A28177, 0.25 μg/mL, 1:4000 dilution). An 86 kDa band corresponding to EZH2 was observed across all cell lines tested.



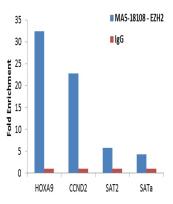
EZH2 Antibody (MA5-18108)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. Hep G2 cells were transfected with EZH2 siRNA and reduction of signal was observed in Western Blot using EZH2 Monoclonal Antibody (144CT2.1.1.5) (Product # MA5-18108). {KD}



EZH2 Antibody (MA5-18108) in ICC/IF

Immunofluorescence analysis of EZH2 was performed using 70% confluent log phase HCT 116 cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with EZH2 Mouse Monoclonal Antibody (144CT2. 1.1.5) (Product # MA5-18108) at 1:50 dilution in 0.1% BSA, incubated at 4 degree Celsius overnight and then labeled with Goat anti-Mouse IgG (H+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 ProLong™ Diamond Antifade Mountant with DAPI (Product # P36962). F-actin (Panel c: red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing nucleus localization. Panel e represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.



EZH2 Antibody (MA5-18108)

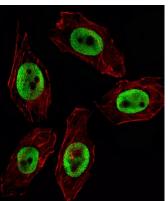
Antibody specificity was demonstrated by detection of enrichment of the target protein at specific gene loci. Chromatin Immunoprecipitation (ChIP) was performed using Anti-EZH2 monoclonal antibody (Product # MA5-18108) using PCR primer pairs over the promoters of HOXA9, CCND2 (positive) and SAT2 satellite repeats, SATa satellite alpha (negative). {RE}

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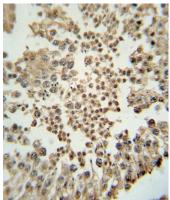
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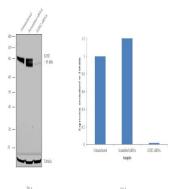
EZH2 Antibody (MA5-18108) in ICC/IF

Immunofluorescent analysis of EZH2 showing staining in the nucleus of U-251 cells using an EZH2 monoclonal antibody (Product # MA5-18108) followed by detection using a fluorescent conjugated secondary antibody (green). Cytoplasmic actin was stained with a fluorescent red phalloidin (7units/mL, 1 h at 37°C).



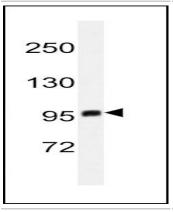
EZH2 Antibody (MA5-18108) in IHC (P)

Immunohistochemistry analysis of EZH2 in formalin-fixed, paraffin-embedded human testis tissue using an EZH2 monoclonal antibody (Product # MA5-18108) followed by peroxidase conjugation of the secondary antibody and DAB staining.



EZH2 Antibody (MA5-18108) in WB

Knockdown of EZH2 was achieved by transfecting Hep G2 cells with EZH2 specific siRNAs (Silencer® select Product # s4918, s4917). Western blot analysis (Fig. a) was performed using whole cell extracts from the EZH2 knockdown cells (lane 3), non-specific scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blots were probed with EZH2 Monoclonal Antibody (144CT2.1.1.5) (Product # MA5-18108, 1:2000 dilution) and Goat anti-Mouse IgG (H+L) SuperclonalTM Secondary Antibody, HRP conjugate (Product # A28177, 0.25 μg/mL, 1:4000 dilution). Densitometric analysis of this western blot is shown in histogram (Fig. b). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to EZH2.



EZH2 Antibody (MA5-18108) in WB

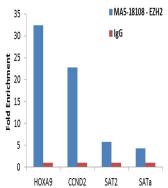
Western blot analysis of EZH2 in T47D cell lysate (35 µg/lane) using an EZH2 monoclonal antibody (Product # MA5-18108).

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EZH2 Antibody (MA5-18108) in ChIP

Enrichment of endogenous EZH2 protein at specific gene loci using Anti-EZH2 Antibody: Chromatin Immunoprecipitation (ChIP) was performed using Anti-EZH2 mouse monoclonal antibody (Product # MA5-18108, 8 ul) on sheared chromatin from 2 million HCT 116 cells using the MAGnify ChIP System (Product # 49-2024). Normal Rabbit IgG was used as a negative IP control. The purified DNA was analyzed by qPCR with PCR primer pairs over the promoters of HOXA9, CCND2 (positive) and SAT2 satellite repeats and SATa satellite alpha (negative). Data is presented as fold enrichment of the antibody signal versus the negative control IgG using the comparative CT method.

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PubMed References	For EZH2 Monoclonal Antibody (144CT2.1.1.5)
1 Immunocytochemistr	y References
Species / Dilution	Summary
Human / 1:20	MA5-18108 was used in Immunocytochemistry to provide a global quantitative measurement of LLPS properties of chromatin-associated proteins and higher-order chromatin structure.
	Genome biology (2021; 22:) "Quantifying the phase separation property of chromatin-associated proteins under physiological conditions using an anti-1,6-hexanediol index." Author(s):Shi M,You K,Chen T,Hou C,Liang Z,Liu M,Wang J,Wei T,Qin J,Chen Y,Zhang MQ,Li T PubMed Article URL:http://dx.doi.org/10.1186/s13059-021-02456-2

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