

CENPA Polyclonal Antibody

Catalog NumberPA5-17194

Product data sheet

Details		Species Reactivity	
Size	100 µL	Species reactivity	Human, Rat
Host/Isotope	Rabbit / IgG	Tested Applications	Dilution *
Class	Polyclonal		
Type	Antibody	Western Blot (WB)	1:1,000
Immunogen	Synthetic peptide corresponding to human CENP-A protein	Immunocytochemistry (ICC/IF)	1:200
		* 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.	
Conjugate	Unconjugated		
Form	Liquid		
Concentration	238 µg/mL		
Purification	Antigen affinity chromatography		
Storage buffer	0.01M HEPES, pH 7.5, with 0.15M NaCl, 100µg/mL BSA, 50% glycerol		
Contains	no preservative		
Storage Conditions	-20°C		

Product specific information

It is not recommended to aliquot this antibody. This antibody is not cross-reactive with other histone proteins, including Histone H3.

Background/Target Information

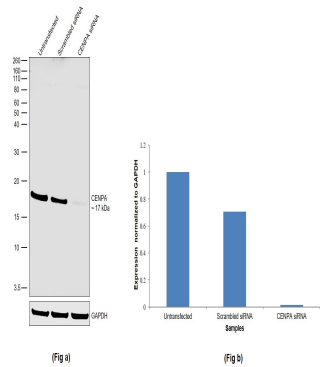
Centromeres are the differentiated chromosomal domains that specify the mitotic behavior of chromosomes. CENPA encodes a centromere protein which contains a histone H3 related histone fold domain that is required for targeting to the centromere. CENPA is proposed to be a component of a modified nucleosome or nucleosome-like structure in which it replaces 1 or both copies of conventional histone H3 in the (H3-H4)<sub>2</sub> tetrameric core of the nucleosome particle.

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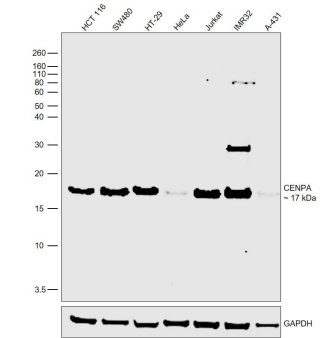


CENPA Antibody (PA5-17194)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. HT-29 cells were transfected with CENPA siRNA and decrease in signal intensity was observed in Western Blot application using Anti-CENPA Polyclonal Antibody (Product # PA5-17194). {KD}

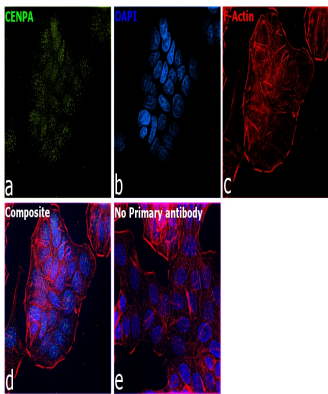
CENPA Antibody (PA5-17194) in WB

Western blot was performed using Anti-CENPA Polyclonal Antibody(Product # PA5-17194) and a 17kDa band corresponding to CENPA was observed across the cell lines tested. Nuclear enriched extracts (30 µg lysate) of HCT 116 (Lane 1), SW480 (Lane 2), HT-29 (Lane 3), HeLa (Lane 4), Jurkat (Lane 5), IMR32 (Lane 6) and A-431 (Lane 7) were electrophoresed using NuPAGE™ 12% Bis-Tris Protein Gel (Product # NP0342BOX). Resolved proteins were then transferred onto a Nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (1:1000) and detected by chemiluminescence with Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036,1:4000) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005).



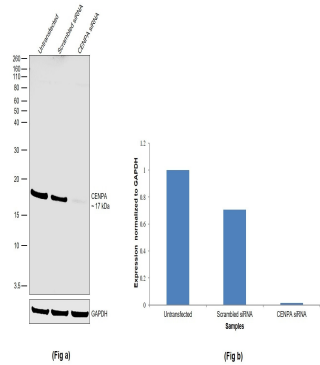
CENPA Antibody (PA5-17194) in ICC/IF

Immunofluorescence analysis of CENPA was performed using 70% confluent log phase HT-29 cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 2% BSA for 45 minutes at room temperature. The cells were labeled with CENPA Polyclonal Antibody (Product # PA5-17194) at 1:200 in 0.1% BSA, incubated at 4 degree celsius overnight and then labeled with Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 (Product # A32790), (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 Centromere localization. Panel e represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.



CENPA Antibody (PA5-17194) in WB

Knockdown of CENPA was achieved by transfecting HT-29 with CENPA specific siRNAs (Silencer® select Product # S2907, S2906). Western blot analysis (Fig. a) was performed using Nuclear enriched extracts from the CENPA knockdown cells (lane 3), non-targeting scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blot was probed with CENPA Polyclonal Antibody (Product # PA5-17194, 1:1000) and Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:4000). 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 CENPA.



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