Anti-CENPA Antibody [PSH0-09]

HA721262

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell
Molecular Wt:	Predicted band size: 16 kDa
Clone number:	PSH0-09

Description: Centromere protein A, also known as CENPA, is a protein which in humans is encoded by the CENPA gene. CENPA is a histone H3 variant which is the critical factor determining the kinetochore position(s) on each chromosome in most eukaryotes including humans. CENPA is a protein which epigenetically defines the position of the centromere on each chromosome, determining the position of kinetochore assembly and the final site of sister chromatid cohesion during mitosis. The CENPA protein is a histone H3 variant which replaces one or both canonical H3 histones in a subset of nucleosomes within centromeric chromatin. CENPA has the greatest sequence divergence of the histone H3 variants, with just 48% similarity to canonical histone H3, and has a highly diverged N-terminal tail that lacks many well characterised histone modification sites including H3K4, H3K9 and H3K27. Unusually for a histone, CENPA nucleosomes are not loaded together with DNA replication and are loaded at different cell cycle stages in different organisms: G1 phase in human, M phase in drosophila, G2 in S. pombe. To orchestrate this specialised loading there are CENPA-specific histone chaperones: HJURP in human, CAL1 in drosophila and Scm3 in S. pombe. In most eukaryotes CENPA is loaded into large domains of highly repetitive satellite DNA. The position of CENPA within satellite DNA are heritable at the protein level through a purely epigenetic mechanism. This means that the position of CENPA protein binding to the genome is copied upon cell division to the two daughter cells independent of the underlying DNA sequence. Under circumstances in which CENPA is lost from a chromosome a fail-safe mechanism has been described in human cells in which CENPB recruits CENPA via a satellite DNA binding domain to repopulate the centromere with CENPA nucleosomes. CENPA interacts directly with the inner kinetochore through proteins including CENPC and CENPN. Through this interaction the microtubules are able to accurately segregate chromosomes during mitosis.

Immunogen:	Synthetic peptide within human CENPA aa 21-70 / 140.
Positive control:	Hela cell lysate, A375 cell lysate, HeLa.
Subcellular location:	Nucleus, Chromosome, centromere, kinetochore.
Database links:	SwissProt: P49450 Human
Recommended Dilutions:	
WB	1:1,000
IF-Cell	1:100
Storage Buffer:	PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.
Purity:	Protein A affinity purified.

Images

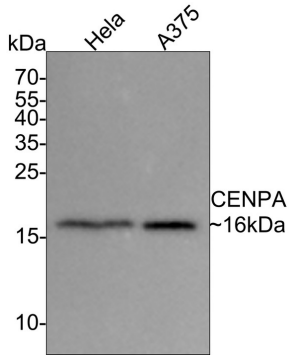

Fig1: Western blot analysis of CENPA on different lysates with Rabbit anti-CENPA antibody (HA721262) at 1/1,000 dilution.

Lane 1: Hela cell lysate
Lane 2: A375 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 16 kDa
Observed band size: 16 kDa

Exposure time: 5 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721262) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

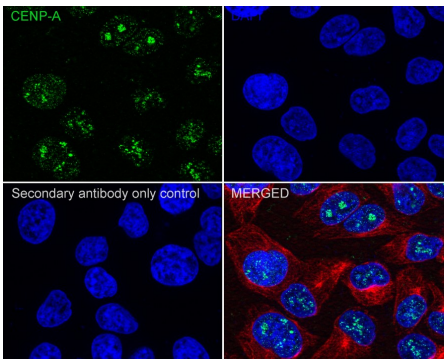

Fig2: Immunocytochemistry analysis of HeLa cells labeling CENPA with Rabbit anti-CENPA antibody (HA721262) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 2% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-CENPA antibody (HA721262) at 1/100 dilution in 0.1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Background References

1. Wang Q et al. CENPA promotes clear cell renal cell carcinoma progression and metastasis via Wnt/β-catenin signaling pathway. J Transl Med. 2021 Oct
2. Han J et al. CENPA is one of the potential key genes associated with the proliferation and prognosis of ovarian cancer based on integrated bioinformatics analysis and regulated by MYBL2. Transl Cancer Res. 2021 Sep