Anti-CENPB Antibody [A5F5]

HA600070

Product Type:	Mouse monoclonal IgG2a, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IHC-P, IP
Molecular Wt:	65 kDa
Clone number:	A5F5

Description: This gene product is a highly conserved protein that facilitates centromere formation. It is a DNA-binding protein that is derived from transposases of the pogo DNA transposon family. It contains a helix-loop-helix DNA binding motif at the N-terminus, and a dimerization domain at the C-terminus. The DNA binding domain recognizes and binds a 17-bp sequence (CENP-B box) in the centromeric alpha satellite DNA. This protein is proposed to play an important role in the assembly of specific centromere structures in interphase nuclei and on mitotic chromosomes. It is also considered a major centromere autoantigen recognized by sera from patients with anti-centromere antibodies.

Immunogen: Recombinant protein within human CENPB aa 1-200 / 599.

Positive control: Hela cell lysate, A549 cell lysate, MCF-7 cell lysate, SK-OV-3, A549, human kidney tissue, human skin tissue.

Subcellular location: Nucleus, centromere.

Database links: SwissProt P07199 Human

Recommended Dilutions:

WB	1:500-1:2,000
IF-Cell	1:50-1:400
IHC-P	1:100-1:500
IP	Use at an assay dependent concentration.

Storage Buffer: 1*TBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein G affinity purified.

Images

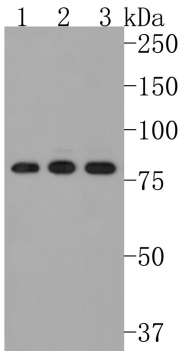

Fig1: Western blot analysis of CENPB on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA60, 1/500) was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:20,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: Hela cell lysate
Lane 2: A549 cell lysate
Lane 3: MCF-7 cell lysate

Predicted band size: 65 kDa
Observed band size: 80 kDa

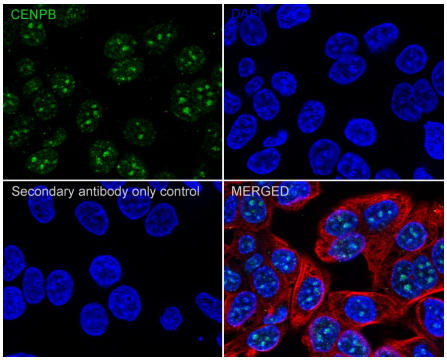

Fig2: Immunocytochemistry analysis of SK-OV-3 cells labeling CENPB with Mouse anti-CENPB antibody (HA600070) at 1/400 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Mouse anti-CENPB antibody (HA600070) at 1/400 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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 (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

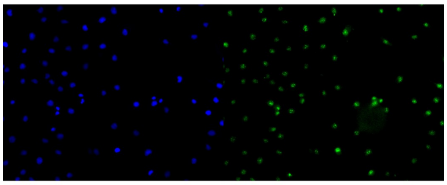

Fig3: ICC staining of CENPB in A549 cells (green). Methanol fixed cells were blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (HA600070, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Mouse IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

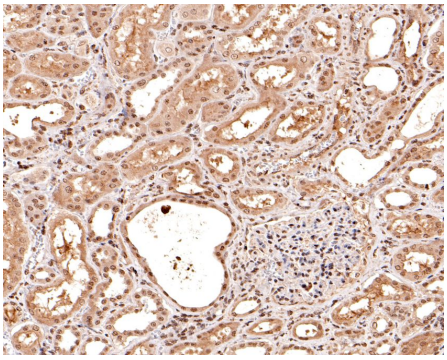

Fig4: Immunohistochemical analysis of paraffin-embedded human kidney tissue using anti-CENPB antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA600070, 1/400) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

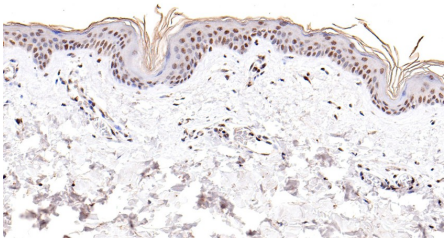

Fig5: Immunohistochemical analysis of paraffin-embedded human skin tissue using anti-CENPB antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA600070, 1/100) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

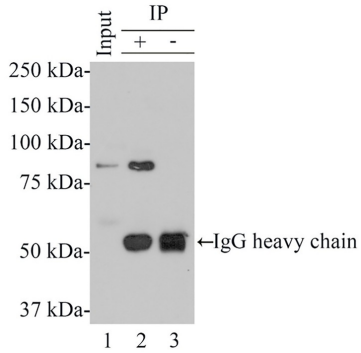

Fig6: CENPB was immunoprecipitated from 0.4 mg Hela whole cell lysates with HA600070 at 2 µg/mL. Western blot was performed from the immunoprecipitate using HA600070 at 1/500 dilution for 45 minutes at room temperature. Goat anti-Mouse IgG - HRP Secondary Antibody (HA1006) was used at 1:100,000 dilution for 30 minutes at room temperature.

Lane 1: Hela whole cell lysates at 4 µg;
Lane 2: CENPB (HA600070) IP in Hela whole cell lysates;
Lane 3: Mouse IgG instead of CENPB (HA600070) in Hela whole cell lysates.

Predicted band size: 65 kDa
Observed band size: 85 kDa

Exposure time: 2 minutes;

8% SDS-PAGE gel.

Background References

1. Otake K. et. al. CENP-B creates alternative epigenetic chromatin states permissive for CENP-A or heterochromatin assembly. J Cell Sci. 2020 Aug
2. Ohzeki JI. et. al. Human artificial chromosome: Chromatin assembly mechanisms and CENP-B. Exp Cell Res. 2020 Apr