Anti-CENPC Antibody [JG36-15]

ET7108-24

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC, IP
Molecular Wt:	107 kDa
Clone number:	JG36-15

Description: A replicated chromosome includes two kinetochores that control chromosome segregation during mitosis. The evolutionarily conserved Centromere Protein-C, CENP-C, is a kinetochore assembly protein. CENP-C is located on the fibers of the kinetochore and constitutes a kinetochore organizing center that tightly associates with DNA. CENP-C is necessary for the formation of a functional centromere, which indicates that CENP-C is important for mitotic progression. In addition, CENP-C is lost from centromeres during herpes simplex virus 1 infection, causing substantial structural changes in the kinetochore, which suggests that the structure of CENP-C is regulated during the cell cycle.

Immunogen: Recombinant protein within Human CENPC aa 360-540 / 943.

Positive control: HL-60 cell lysate, Daudi cell lysate, K562 cell lysate, SH-SY5Y, mouse spleen tissue, rat heart tissue, Hela.

Subcellular location: Nucleus, kinetochore, centromere.

Database links: SwissProt: Q03188 Human | P49452 Mouse
Entrez Gene: 305270 Rat

Recommended Dilutions:

WB	1:500-1:2,000
IF-Cell	1:50-1:200
IHC-P	1:50-1:200
FC	1:50-1:200
IP	Use at an assay dependent concentration.

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Images

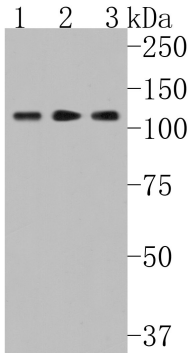

Fig1: Western blot analysis of CENPC on different lysates with Rabbit anti-CENPC antibody (ET7108-24) at 1/500 dilution.

Lane 1: HL-60 cell lysate
Lane 2: Daudi cell lysate
Lane 3: K562 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 107 kDa
Observed band size: 107 kDa

Exposure time: 2 minutes;

10% 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 (ET7108-24) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

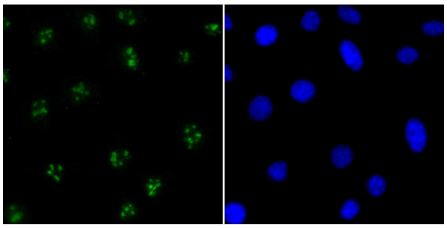

Fig2: Immunocytochemistry analysis of SH-SY5Y cells labeling CENPC with Rabbit anti-CENPC antibody (ET7108-24) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-CENPC antibody (ET7108-24) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃.Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

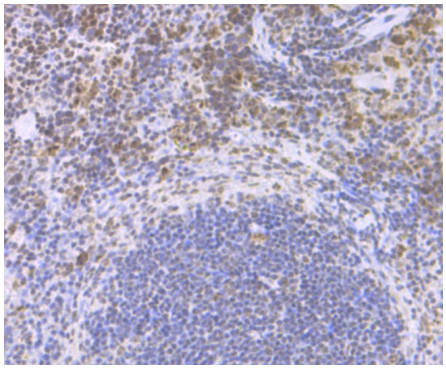

Fig3: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-CENPC antibody (ET7108-24) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4)) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET7108-24) at 1/50 dilution for 0.5 hour 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.

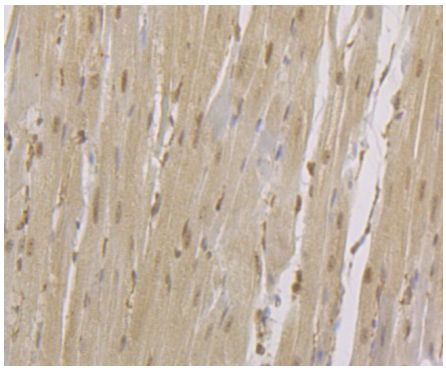

Fig4: Immunohistochemical analysis of paraffin-embedded rat heart tissue with Rabbit anti-CENPC antibody (ET7108-24) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4)) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET7108-24) at 1/50 dilution for 0.5 hour 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.

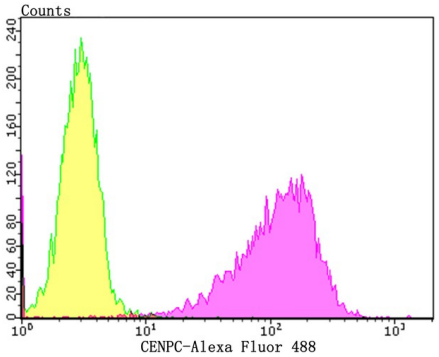

Fig5: Flow cytometric analysis of CENPC was done on Hela cells. The cells were fixed, permeabilized and stained with the primary antibody (ET7108-24, 1/50) (purple). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; yellow).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Gopalakrishnan S et al. DNMT3B interacts with constitutive centromere protein CENP-C to modulate DNA methylation and the histone code at centromeric regions. Hum Mol Genet 18:3178-3193 (2009).
2. Gascoigne K E et al. Induced ectopic kinetochore assembly bypasses the requirement for CENP-A nucleosomes. Cell 145:410-422 (2011).