abcam

Product datasheet

Anti-CENPE antibody [1H12] ab5093

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Overview

Product name	Anti-CENPE antibody [1H12]
Description	Mouse monoclonal [1H12] to CENPE
Host species	Mouse
Tested applications	**Suitable for:** ICC/IF, WB, Flow Cyt
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant full length protein (Human).
Positive control	Any human cell line should be suitable as a positive control. Kinetochore staining only visible in mitosis.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.1% Sodium azide Constituent: PBS
Purification notes	Purified from tissue culture supernatant via ion exchange chromatography (>95% total IgG).
Clonality	Monoclonal
Clone number	1H12
Myeloma	Sp2/0
Isotype	IgG1
Light chain type	kappa

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab5093 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★★ (4)	Use at an assay dependent concentration.
WB		Use a concentration of 0.5 - 1 µg/ml. Predicted molecular weight: 312 kDa. Only suitable for WB if IP is performed first.
Flow Cyt		Use 1µg for 10^6 cells. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Target

Function Essential for the maintenance of chromosomal stability through efficient stabilization of microtubule capture at kinetochores. Plays a key role in the movement of chromosomes toward the metaphase plate during mitosis. Is a slow plus end-directed motor whose activity is essential for metaphase chromosome alignment. Couples chromosome position to microtubule depolymerizing activity. The highly processive microtubule-dependent motor activity of CENPE serves to power chromosome congression and provides a flexible, motile tether linking kinetochores to dynamic spindle microtubules. Necessary for the mitotic checkpoint signal at individual kinetochores to prevent aneuploidy due to single chromosome loss. Required for the efficient recruitment of BUBR1, MAD1 and MAD2 to attached and newly unattached kinetochores. Stimulates mammalian BUBR1 kinase activity. Accumulates just before mitosis at the G2 phase of the cell cycle.

Involvement in disease Microcephaly 13, primary, autosomal recessive

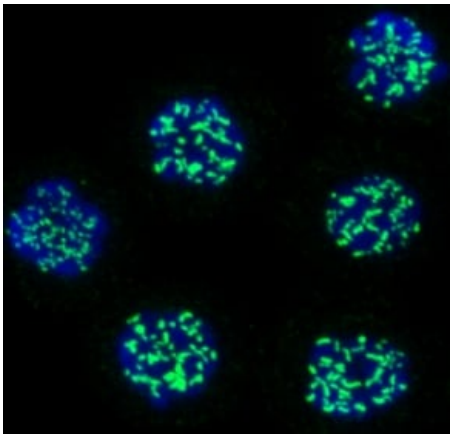
Sequence similarities Belongs to the TRAFAC class myosin-kinesin ATPase superfamily. Kinesin family.
Contains 1 kinesin motor domain.

Domain The protein is composed of a N-terminal kinesin-motor domain involved in the chromosome movements, a long coil-coiled region involved in the homodimerization and an inhibitory C-tail involved in autoinhibition of the N-terminal catalytic part.

Post-translational modifications The C-terminal inhibitory domain is phosphorylated. Phosphorylation relieves autoinhibition of the kinetochore motor.
Sumoylated with SUMO2 and SUMO3. The sumoylation mediates the association to the kinetochore.

Cellular localization Chromosome, centromere, kinetochore. Cytoplasm, cytoskeleton, spindle. Associates with kinetochores during congression (as early as prometaphase), relocates to the spindle midzone at anaphase, and is quantitatively discarded at the end of the cell division.

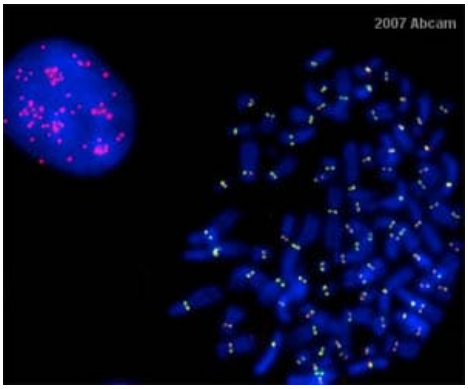
Images



Immunocytochemistry/ Immunofluorescence - Anti-CENPE antibody [1H12] (ab5093)

Kinetochores specific staining of HCT116 cells arrested in G2/M phase by nocodazole treatment. Methanol fixed cells were stained using mouse monoclonal [1H12] antibody to CENP-E ab5093 (green) and DAPI (blue).

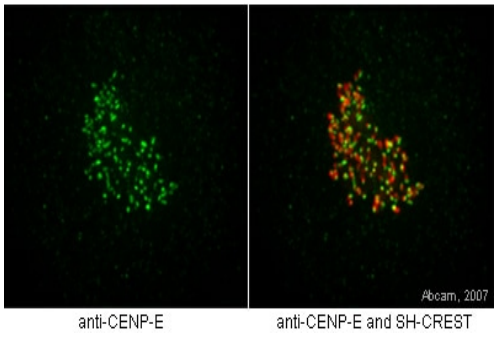
This image was kindly supplied as part of the review submitted by Salvador Rodrigez-Nieto.



Immunocytochemistry/ Immunofluorescence - Anti-CENPE antibody [1H12] (ab5093)

This image is courtesy of an Abreview submitted by Dr Beth Sullivan

ab5093 at 1/500 staining human fibrosarcoma (HT1080) cells by ICC/IF. The cells were treated with 0.1-0.2ug/mL colcemid for 45-60 minutes, then swollen in hypotonic buffer for 8 minutes and centrifuged onto glass slides. Cells were blocked in 1X PBS + 1% BSA + 0.5% Triton X-100 (blocking buffer) for 30 minutes at room temperature. The antibodies were diluted 1/300-1/500 in blocking buffer and incubated overnight at 4 degrees C. ab5093 was detected with Alexa Fluor 488-donkey anti-mouse for 1-2 hours at room temperature.

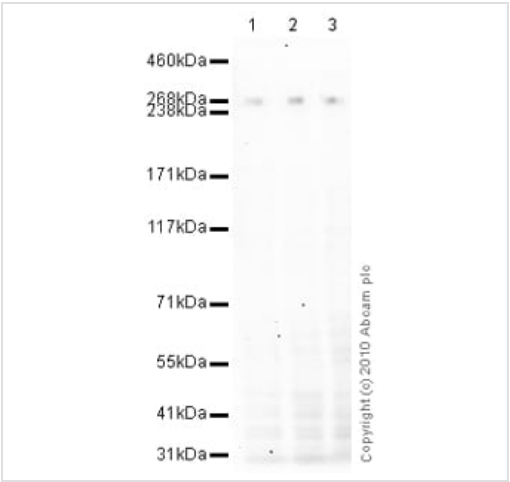


Immunocytochemistry/ Immunofluorescence - Anti-CENPE antibody [1H12] (ab5093)

This image is courtesy of Scott Slattery and Mike Mancini

HeLa cells were stained with ab5093, anti-CENPE (in green) in panel one, and with ab5093 and SH-CREST (red), which stains the centromeres, in panel 2. Fix cells for 30 minutes on ice in 4% formaldehyde in PEM. Quench autofluorescence 2 x 5 minutes with 1 mg/ml Na borohydride or 100 mM ammonium chloride in PEM. Permeablize 30 minutes with 0.5% TX-100 in PEM. Block 30 minutes in 5% milk in TBST. Primary antibody overnight at 4oC diluted 1/250 in 5% milk in TBST. Secondary antibody was incubated for 1 hour at RT diluted in 5% milk in TBST. Post-fix 20 minutes on ice in 4% formaldehyde in PEM. Quench autofluorescence 2 x 5 minutes with ammonium chloride in PEM. Counterstain with DAPI in TBST. Mount with ProLong Gold antifade reagent from Invitrogen. Notes: Ample washing between each step. TBST = Tris buffered saline + 0.1% Tween. PEM = 80 mM K-

PIPES, pH 6.8, 5 mM EGTA, 2 mM MgCl2.



Western blot - Anti-CENPE antibody [1H12] (ab5093)

All lanes : Anti-CENPE antibody [1H12] (ab5093) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate
Lane 3 : HEK293 (Human embryonic kidney cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

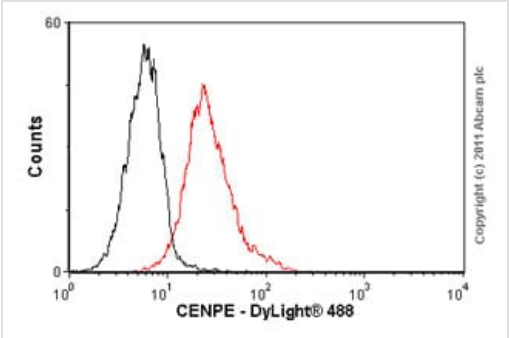
Secondary
All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed (ab97040) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 312 kDa
Observed band size: 270 kDa

Exposure time: 20 minutes



Flow Cytometry - Anti-CENPE antibody [1H12] (ab5093)

Overlay histogram showing HeLA cells stained with ab5093 (red line). The cells were fixed with 100% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab5093, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was Mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

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