abcam

Product datasheet

Anti-CENPF antibody ab5

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Overview

Product name	Anti-CENPF antibody
Description	Rabbit polyclonal to CENPF
Host species	Rabbit
Tested applications	**Suitable for:** ICC, Flow Cyt, ICC/IF, IP, IHC-P, WB
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Fusion protein with C-terminus of CENP-F (Human).
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. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
Storage buffer	pH: 7.4 Preservative: 0.05% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine)
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee	Our **Abpromise guarantee** covers the use of ab5 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	★★★★☆ (1)	Use at an assay dependent concentration.
Flow Cyt	★★★★★ (1)	Use at an assay dependent concentration. **ab171870** - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★☆ (5)	1/400 - 1/750.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration.
WB		1/1500. Detects a band of approximately 330 kDa (predicted molecular weight: 330 kDa). Block membranes for 1 hour with 5% nonfat dry milk/TBS-T.

Target

Function

Required for kinetochore function and chromosome segregation in mitosis. Required for kinetochore localization of dynein, LIS1, NDE1 and NDEL1. Regulates recycling of the plasma membrane by acting as a link between recycling vesicles and the microtubule network though its association with STX4 and SNAP25. Acts as a potential inhibitor of pocket protein-mediated cellular processes during development by regulating the activity of RB proteins during cell division and proliferation. May play a regulatory or permissive role in the normal embryonic cardiomyocyte cell cycle and in promoting continued mitosis in transformed, abnormally dividing neonatal cardiomyocytes. Interaction with RB directs embryonic stem cells toward a cardiac lineage. Involved in the regulation of DNA synthesis and hence cell cycle progression, via its C-terminus. Has a potential role regulating skeletal myogenesis and in cell differentiation in embryogenesis. Involved in dendritic cell regulation of T-cell immunity against chlamydia.

Involvement in disease

Stromme syndrome

Sequence similarities

Belongs to the centromere protein F family.

Developmental stage

Gradually accumulates during the cell cycle, reaching peak levels in G2 and M phase, and is rapidly degraded upon completion of mitosis.

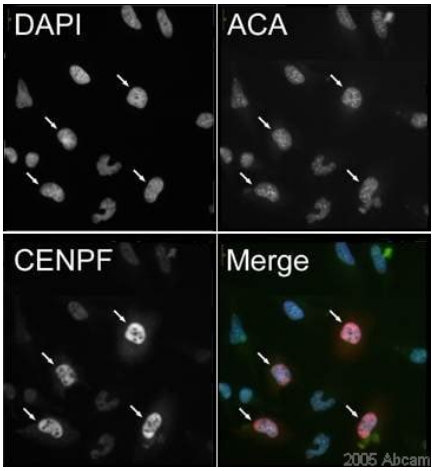
Post-translational modifications

Hyperphosphorylated during mitosis.

Cellular localization

Cytoplasm, perinuclear region. Nucleus matrix. Chromosome, centromere, kinetochore. Cytoplasm, cytoskeleton, spindle. Relocalizes to the kinetochore/centromere (coronal surface of the outer plate) and the spindle during mitosis. Observed in nucleus during interphase but not in the nucleolus. At metaphase becomes localized to areas including kinetochore and mitotic apparatus as well as cytoplasm. By telophase, is concentrated within the intracellular bridge at either side of the mid-body.

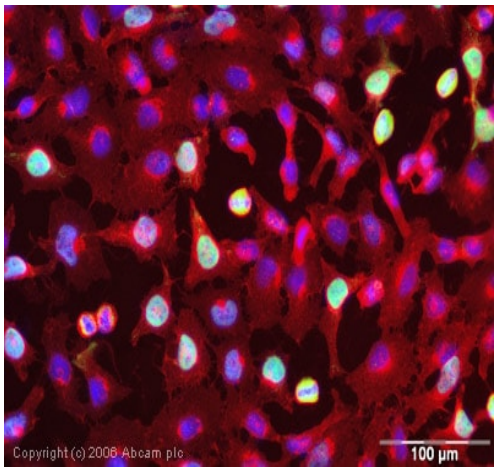
Images



Immunocytochemistry/ Immunofluorescence - Anti-CENPF antibody (ab5)

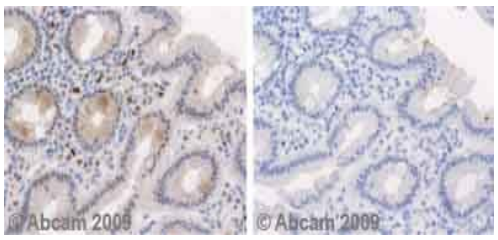
This image is courtesy of Kirk McManus, University of British Columbia

HeLa cells were labelled with anti-ACA and anti-CENPF (ab5). ab5 was used at a working dilution of 1/400. This image demonstrates the dramatic increase in fluorescence that occurs late in G2 cells (indicated by arrows). In the final panel DAPI is pseudo-coloured blue, while ACA and CENPF are coloured green and red respectively. 40x magnification.



Immunocytochemistry/ Immunofluorescence - Anti-CENPF antibody (ab5)

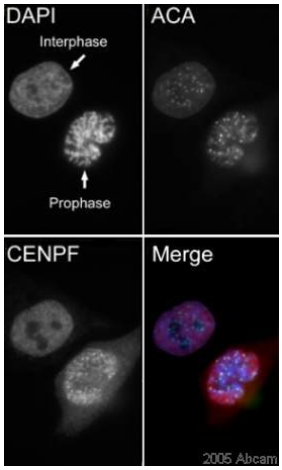
ICC/IF image of ab5 stained human HeLa cells. The cells were methanol fixed (5 min) and incubated with the antibody (ab5, 1µg/ml) for 1h at room temperature. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Image-iT^{TM} FX Signal Enhancer was used as the primary blocking agent, 5% BSA (in TBS-T) was used for all other blocking steps. DAPI was used to stain the cell nuclei (blue). Alexa Fluor® 594 WGA was used to label plasma membranes (red).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CENPF antibody (ab5)

Ab5 staining Human normal colon tissue. Staining is localised to nuclear compartment.
Left panel: with primary antibody at 4 ug/ml. Right panel: isotype control.
Sections were stained using an automated system DAKO Autostainer Plus , at room temperature: sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer EDTA pH 9.0 in a DAKO PT Link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako envision flex amplification kit for 30 minutes. Colorimetric detection

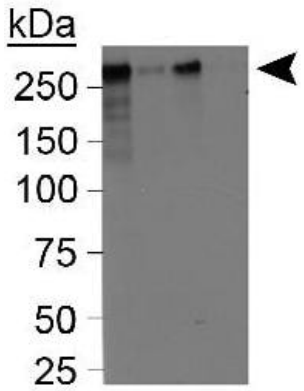
was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be required.



Immunocytochemistry/ Immunofluorescence - Anti-CENPF antibody (ab5)

This image is courtesy of Kirk McManus, University of British Columbia

Interpahse and Prophase HeLa cells were labelled with anti-ACA and anti-CENPF (ab5). ab5 was used at a working dilution of 1/400. This image emphasizes the redistribution of CENPF from the nuclear matrix during late G2 following entry into the initial stages of mitosis (see the accompanying image). Distinct punctate CENPF patterns proximally located in relation to the centromeres can be seen. In the final panel DAPI is pseudo-coloured blue, while ACA and CENPF are green and red respectively. 100x magnification.



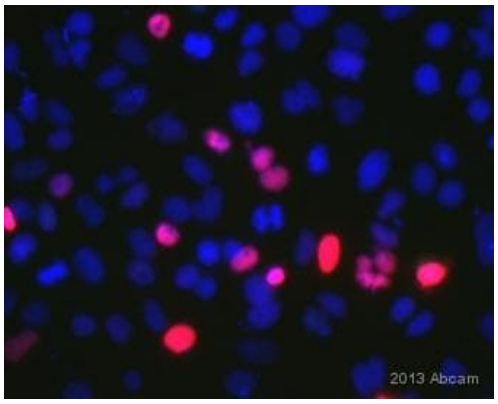
Western blot - Anti-CENPF antibody (ab5)

All lanes : Anti-CENPF antibody (ab5) at 1/1500 dilution

Lane 1 : Mitotic HeLa Lysate
Lane 2 : Asynchronous HeLa Lysate
Lane 3 : 50gamma Mitotic HeLa Lysate
Lane 4 : 50gamma Asynchronous HeLa Lysate

Lysates/proteins at 25 µg per lane.

Predicted band size: 330 kDa
Observed band size: 310 kDa



Immunocytochemistry/ Immunofluorescence - Anti-CENPF antibody (ab5)

This image is courtesy of an Abreview submitted by Dr. Stuart Rulten.

Paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized A549 (human lung carcinoma cell line) cells stained for CENPF (red) using ab5 at 1/500 dilution in ICC/IF.). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 555) at 1/500 dilution was used as the secondary antibody.

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