abcam

Product datasheet

Anti-HEC1 antibody [9G3] ab3613

★★★★★ 11 Abreviews 74 References 6 Images

Overview

Product name	Anti-HEC1 antibody [9G3]
Description	Mouse monoclonal [9G3] to HEC1
Host species	Mouse
Tested applications	**Suitable for:** Flow Cyt, IP, WB, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human, Pig, African green monkey, Chinese hamster
Immunogen	Recombinant protein encoding amino acids 56-642 of human HEC 1 purified from E. coli.
Positive control	HeLa whole cell lysate.
General notes	This product was changed from ascites to tissue culture supernatant on 17th September 2018. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team.

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: 7.40 Constituent: 0.0268% PBS
Purity	Protein G purified
Purification notes	Purified from TCS
Clonality	Monoclonal
Clone number	9G3
Myeloma	unknown
Isotype	IgG2a
Light chain type	unknown

Applications

Our Abpromise guarantee covers the use of **ab3613** 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
Flow Cyt		Use 1µg for 10^6 cells. ab170191 - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.
IP		Use at an assay dependent concentration.
WB	★★★★★	1/1000. Detects a band of approximately 80 kDa (predicted molecular weight: 74 kDa).
ICC/IF	★★★★★	Use at an assay dependent concentration.

Target

Function
Acts as a component of the essential kinetochore-associated NDC80 complex, which is required for chromosome segregation and spindle checkpoint activity. Required for kinetochore integrity and the organization of stable microtubule binding sites in the outer plate of the kinetochore.

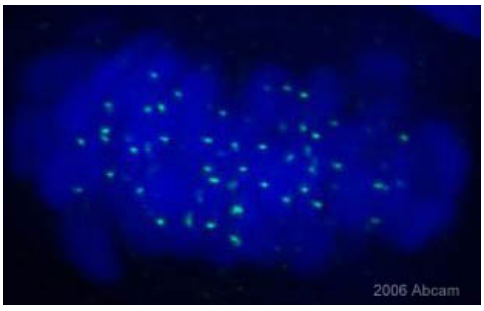
Sequence similarities
Belongs to the NDC80/HEC1 family.

Developmental stage
Expression peaks in mitosis.

Post-translational modifications
Phosphorylation begins in S phase of the cell cycle and peaks in mitosis. Phosphorylated by NEK2. May also be phosphorylated by AURKA and AURKB.

Cellular localization
Nucleus. Chromosome > centromere > kinetochore. Localizes to kinetochores from late prophase to anaphase. Localizes specifically to the outer plate of the kinetochore.

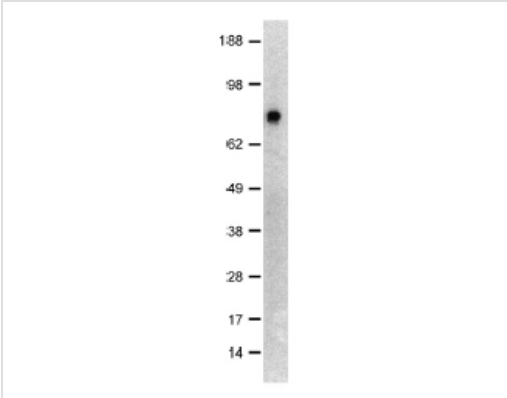
Images



Immunocytochemistry/ Immunofluorescence - Anti-HEC1 antibody [9G3] (ab3613)

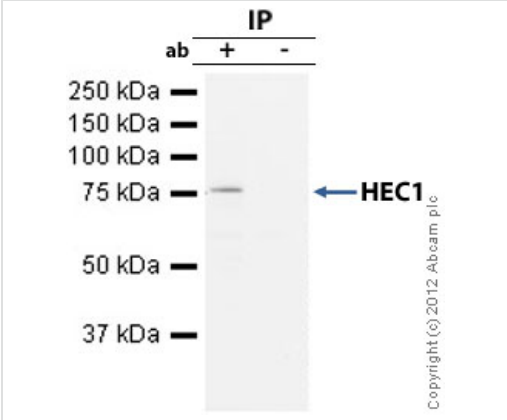
This image is courtesy of an anonymous Abreview

ab3613 at 1/1000 dilution staining HeLa cells by ICC/IF. The cells were formaldehyde fixed and blocked with 5% BSA prior to incubation with the antibody for 2 hours. An Alexa-Fluor ® 488 conjugated goat anti-mouse antibody was used as the secondary.



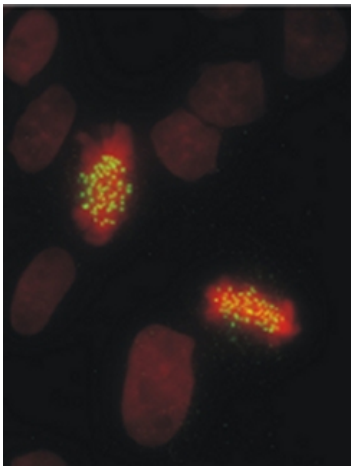
Western blot - Anti-HEC1 antibody [9G3] (ab3613)

ab3613 at a 1/1000 dilution staining ~ 80 kDa HEC 1 in Hela cell lysate (30µg per well) by Western blot. ab3613 at a 1/1000 dilution staining ~ 80 kDa HEC 1 in Hela cell lysate (30µg per well) by Western blot.



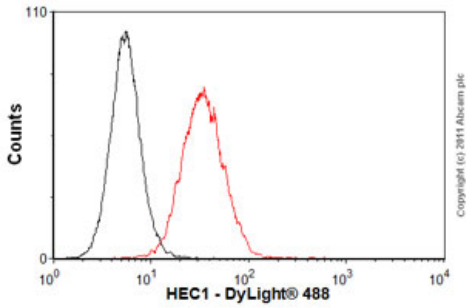
Immunoprecipitation - Anti-HEC1 antibody [9G3] (ab3613)

HEC1 was immunoprecipitated using 0.5mg Hela whole cell extract, 10µg of Mouse monoclonal to HEC1 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{o}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab3613.
Secondary: Goat polyclonal to mouse IgG light chain specific (HRP) at 1/5000 dilution.
Band: Band: 76kDa: HEC1.



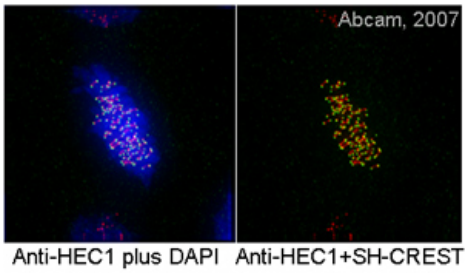
Immunocytochemistry/ Immunofluorescence - Anti-HEC1 antibody [9G3] (ab3613)

Anti-Hec1 antibody (ab3613) labels the kinetochores of mitotic cells in LLCPK1 (Sus scrofa kidney epithelial cell line) cell lines. Merge shows an overlay of DNA (stained with DAPI, red) and Hec1 (green).
This image was kindly supplied as part of the review submitted by Marko Kallio.



Flow Cytometry - Anti-HEC1 antibody [9G3] (ab3613)

Overlay histogram showing HeLa cells stained with ab3613 (red line). The cells were fixed with 4% paraformaldehyde (10 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 (ab3613, 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 IgG2b [PLPV219] (ab91366, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 80% methanol (5 min) /permeabilized in 0.1% PBS-Tween used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-HEC1 antibody [9G3] (ab3613)

This image is courtesy of Scott Slattery and Mike Mancini

HeLA cells were stained with anti-HEC1 (ab2613; in green) and DAPI (blue) in panel 1, and with anti-HEC1(green) and SH-CREST (red) to stain the centromeres in panel 2. Fix the cells 30 minutes on ice in 4% formaldehyde in PEM. Quench autofluorescence 2 x 5 min. with 1 mg/ml Na borohydride or 100 mM ammonium chloride in PEM. Permeablize 30 min. with 0.5% TX-100 in PEM. Block 30 minutes in 5% milk in TBST. Primary antibody incubated at 1/1000 overnight at 4oC diluted in 5% milk in TBST. Secondary antibody 1 hour at RT diluted in 5% milk in TBST. Post-fix 20 min. on ice in 4% formaldehyde in PEM. Quench autofluorescence 2 x 5 min. 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.

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