abcam

Product datasheet

Anti-HEC1/HEC antibody [9G3] ab3613

11 Abreviews 110 References 8 Images

Overview

Product name	Anti-HEC1/HEC antibody [9G3]
Description	Mouse monoclonal [9G3] to HEC1/HEC
Host species	Mouse
Tested applications	**Suitable for:** Flow Cyt, IP, WB, ICC/IF
Species reactivity	**Reacts with:** Human, Pig
Immunogen	Recombinant fragment corresponding to Human HEC1/HEC aa 56-642. Database link: **O14777**
Positive control	WB: HeLa whole cell lysate. ICC/IF: HeLa cells, LLCPK1 (Sus scrofa kidney epithelial cell line). Flow Cytometry: HeLa cells. IP: HeLa whole cell extract.
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. 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: 7.40 Constituent: 100% PBS
Purity	Protein A purified
Purification notes	Purified from TCS
Clonality	Monoclonal

Clone number	9G3
Myeloma	unknown
Isotype	IgG2a
Light chain type	unknown

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab3613 in the following tested applications.

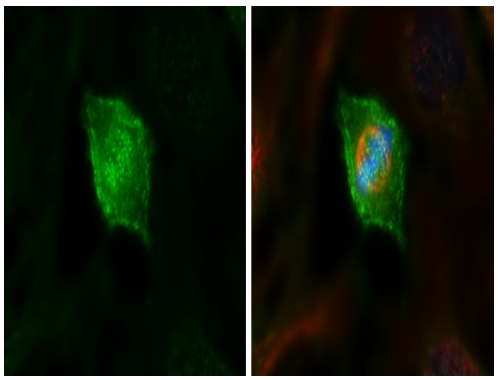
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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. See image legend for details.
WB	★★★★★ **(4)**	1/500 - 1/3000. Detects a band of approximately 80 kDa (predicted molecular weight: 74 kDa).
ICC/IF	★★★★★ **(6)**	1/100 - 1/1000. See protocol in the legend for the HeLa cell image.

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.

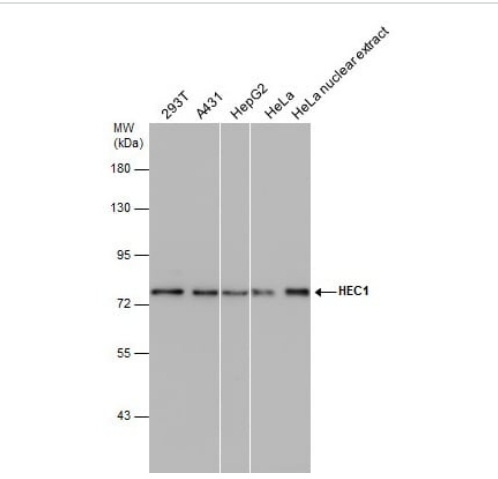
Images



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

Immunocytochemistry/ Immunofluorescence analysis of HeLa cells labeling HEC1/HEC at the kinetochore with ab3613 at 1/500 (green). Cells were fixed in 4% paraformaldehyde at room temperature for 15 minutes. The cells were permeabilized with 0.1% Triton X-100 in PBS at room temperature for 4 minutes. The cells were blocked in 2.5% BSA/PBS at room temperature for 30 minutes. ab3613 was incubated at 4°C overnight. The secondary antibody was a Rabbit IgG antibody (Alexa Fluor 488), 1/2000 (keep from light), room temperature for 1hour. Washing with PBS 3 x 3 minutes. Red: alpha Tubulin 4a, a cytoskeleton marker, stained by an alpha Tubulin 4a antibody.
Blue: Hoechst 33342 staining.

Synchronized condition: Suggest to treat cells with Nocodazole (10ng/ml, 24hr).



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

All lanes : Anti-HEC1/HEC antibody [9G3] (ab3613) at 1/1000 dilution

Lane 1 : 293T whole cell lysate
Lane 2 : A431 whole cell lysate
Lane 3 : HepG2 whole cell lysate
Lane 4 : HeLa whole cell lysate
Lane 5 : HeLa nuclear extract

Lysates/proteins at 30 µg per lane.

Secondary
All lanes : Mouse IgG antibody (HRP) at 1/5000 dilution

Developed using the ECL technique.

Predicted band size: 74 kDa

7.5% gel.

Running condition: 80V, 15min; 140V, 40min.

Transfer condition: Semi-dry, 18 V, 60min (Nitrocellulose

membrane).

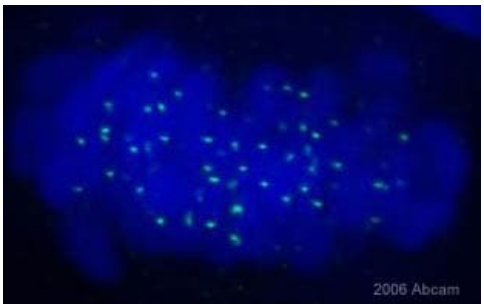
Blocking condition: 5% non-fat milk in TBST, RT, 60min.

Primary antibody incubation: 4°C overnight.

Secondary antibody incubation: Room temperature for 1 hour.

Washing condition: 5 ml TBST, 4 x 5min.

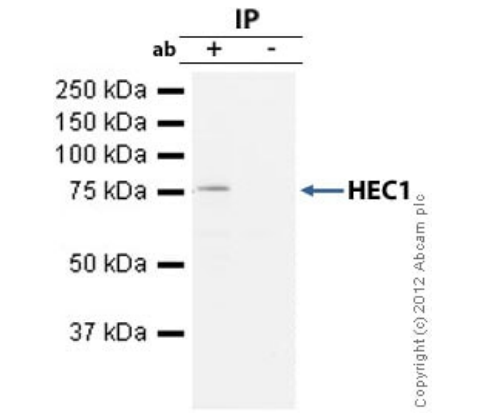
ECL detection.



Immunocytochemistry/ Immunofluorescence - Anti-HEC1/HEC 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.



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

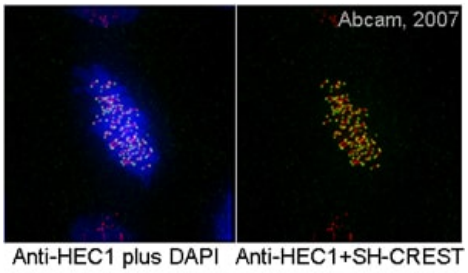
HEC1/HEC was immunoprecipitated using 0.5mg Hela whole cell extract, 10µg of Mouse monoclonal to HEC1/HEC and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

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.

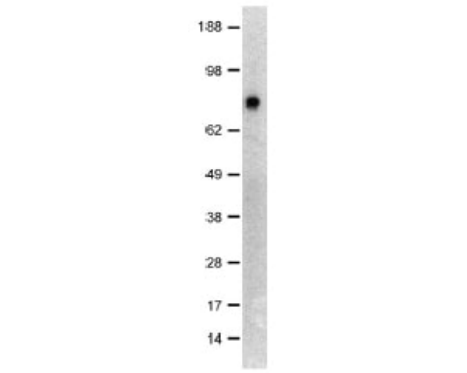
Band: Band: 76kDa: HEC1/HEC.



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

This image is courtesy of Scott Slattery and Mike Mancini

HeLa cells were stained with anti-HEC1/HEC (**ab2613**; in green) and DAPI (blue) in panel 1, and with anti-HEC1/HEC (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.



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

Anti-HEC1/HEC antibody [9G3] (ab3613) at 1/1000 dilution (4? , O/N) + HeLa cell lysate at 30 µg

Secondary

Mouse IgG antibody (HRP) at room temperature for 1 hour at 1/5000 dilution

Predicted band size: 74 kDa

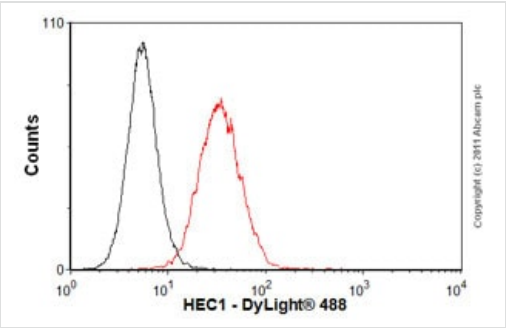
Running conditions: run at 80V for 15 minutes then at 140V for 40 minutes

Transfer conditions: Semi-dry at 18 V for 60min (NC membrane)

Blocking conditions: 5% non-fat milk in TBST, RT, 60min.

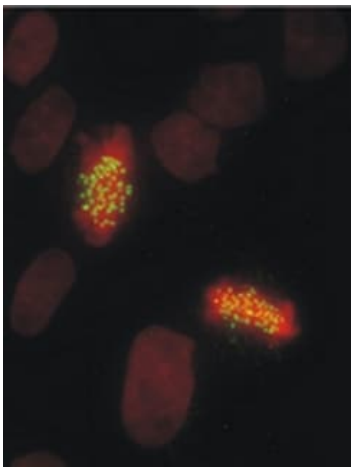
Washing conditions: 5 ml TBST, 4 x 5min

Exposure system used: Trident plus Western HRP Substrate



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

Anti-HEC1/HEC 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/HEC (green).
This image was kindly supplied as part of the review submitted by Marko Kallio.

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