abcam

ab3613

Anti-HEC1/HEC antibody [9G3]

Anti-HEC1/HEC antibody [9G3] (ab3613) is a mouse monoclonal antibody detecting HEC1/HEC in **Western Blot, Flow Cytometry, IP, ICC/IF**. Suitable for **Human, Pig**.

- Over 110 publications
- Trusted since 2003

Key facts

Isotype	IgG2a
Host species	Mouse
Storage buffer	pH: 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Recombinant Fragment Protein within Human NDC80 aa 50 to C-terminus. The exact immunogen used to generate this antibody is proprietary information. Database link O14777
Carrier free	Yes
Clone number	9G3
Purification technique	Affinity purification Protein A
Light chain type	unknown
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Purified from TCS

Reactivity data

IP

Tested

Species	Human
Dilution info	-
Notes	See image legend for details.

Expected

Species	Pig
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	1 µg for 10^6 Cells
Notes	ab170191 - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.

Expected

Species	Pig
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human
Dilution info	1/500 - 1/3000
Notes	-

Expected

Species	Pig
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Tested

Species	Pig
Dilution info	1/100 - 1/1000
Notes	See protocol in the legend for the HeLa cell image.

Species	Human
Dilution info	1/100 - 1/1000
Notes	See protocol in the legend for the HeLa cell image.

Target data

See full target information NDC80

Function	Acts as a component of the essential kinetochore-associated NDC80 complex, which is required for chromosome segregation and spindle checkpoint activity (PubMed:12351790, PubMed:14654001, PubMed:14699129, PubMed:15062103, PubMed:15235793, PubMed:15239953, PubMed:15548592, PubMed:16732327, PubMed:30409912, PubMed:9315664). Required for kinetochore integrity and the organization of stable microtubule binding sites in the outer plate of the kinetochore (PubMed:15548592, PubMed:30409912). The NDC80 complex synergistically enhances the affinity of the SKA1 complex for microtubules and may allow the NDC80 complex to track depolymerizing microtubules (PubMed:23085020). Plays a role in chromosome congression and is essential for the end-on attachment of the kinetochores to spindle microtubules (PubMed:23891108, PubMed:25743205).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C

Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-HEC1/HEC antibody [9G3] (ab3613) is a mouse monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Flow Cyt), Immunoprecipitation (IP), Immunocytochemistry/immunofluorescence (ICC/IF) in Human, Pig samples.

What is the molecular weight of HEC1/HEC?
Anti-HEC1/HEC [9G3] (ab3613) specifically detects a band for HEC1/HEC (UniProt: O14777) at a molecular weight of 74kDa.

Trusted by the scientific community
Anti-HEC1/HEC [9G3] (ab3613) was first used in a scientific publication in 2003 and has been cited over 110 times in peer-reviewed journals.

Reviewed by scientists
Anti-HEC1/HEC [9G3] (ab3613) has over 10 independent reviews from customers.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	HEC1 also known as NDC80 or HEC is an important component of the kinetochore a protein complex importantly involved in chromosome segregation during cell division. It is often referred to as HEC-1 with a molecular mass of approximately 80 kDa. HEC1 is expressed ubiquitously in proliferating cells and it plays an important role in the accurate alignment and segregation of chromosomes during mitosis. The protein is found at centromeres where it is essential for proper attachment of microtubules.
Biological function summary	In the context of cell division HEC1 functions as part of the NDC80 complex a critical ensemble of four proteins that include NUF2 SPC24 and SPC25. This complex is vital for microtubule binding and kinetochore function. HEC1 as a subunit of NDC80 ensures kinetic stability and maintains the structural integrity of the kinetochore during the cell cycle. Its function is necessary for accurate chromosome alignment on the metaphase plate which prevents missegregation and aneuploidy.
Pathways	HEC1 is actively involved in the spindle assembly checkpoint (SAC) a safety mechanism that prevents anaphase onset until all chromosomes are properly aligned. This pathway is important in regulating cell cycle progression and preventing genomic instability. HEC1 also interacts with proteins like BUB1 and

	MAD2 which are integral to the SAC pathway. HEC1’s associations highlight its role in maintaining mitotic accuracy and integrity in dividing cells.
Associated diseases and disorders	HEC1 has a significant connection to cancer. Aberrant expression or malfunction of HEC1 contributes to chromosomal instability a hallmark of cancer progression. Its interactions with oncogenes and tumor suppressor proteins like TP53 can exacerbate this instability leading to unregulated cell division and tumor growth. Additionally HEC1's role in the cell cycle and proliferation links it to disorders like aneuploidies where chromosome number errors occur further implicating its importance in maintaining chromosomal balance.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

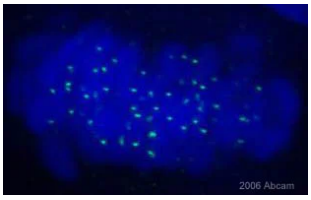
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

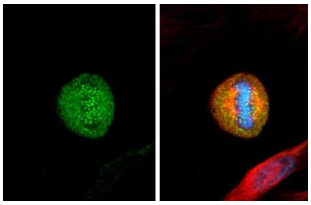
10 product images



This image is courtesy of an anonymous customer review

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

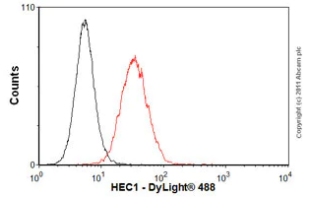
ab3613 at 1/1000 dilution staining HeLa cells by ICC/IF. The cells were formaldehyde fixed and blocked with 5% BSA prior to incubation with the antibody for 2 hours. An Alexa-Fluor ® 488 conjugated goat anti-mouse antibody was used as the secondary.



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

HEC1/HEC Immunocytochemistry/ Immunofluorescence staining of HeLa cells using mouse Anti-HEC1/HEC antibody

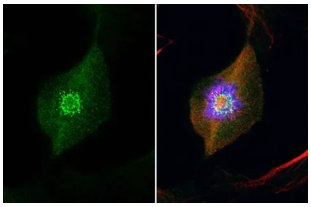
Immunofluorescence analysis of HeLa cells fixed in 4% paraformaldehyde at RT for 15 min labelling Hec1 protein at kinetochore using ab3613 at a 1/1000 dilution (Green). alpha Tubulin, a cytoskeleton marker, stained by alpha Tubulin antibody used at a 1/1000 dilution (Red). DAPI was used for nuclear staining (Blue).



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

HEC1/HEC Flow Cytometry staining using mouse Anti-HEC1/HEC antibody

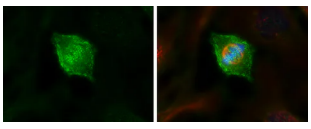
Overlay histogram showing HeLa cells stained with ab3613 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions 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)

HEC1/HEC Immunocytochemistry/ Immunofluorescence staining of HeLa cells using mouse Anti-HEC1/HEC antibody

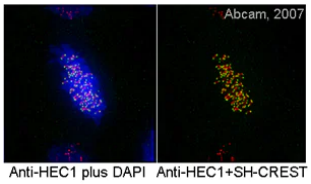
Immunofluorescence analysis of HeLa cells fixed in 4% paraformaldehyde at RT for 15 min labelling Hec1 protein at kinetochore using ab3613 at a 1/500 dilution (Green). beta Tubulin, a cytoskeleton marker, stained by beta Tubulin antibody used at a 1/1000 dilution (Red). DAPI was used for nuclear staining (Blue).



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

HEC1/HEC Immunocytochemistry/ Immunofluorescence staining using mouse Anti-HEC1/HEC antibody

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).

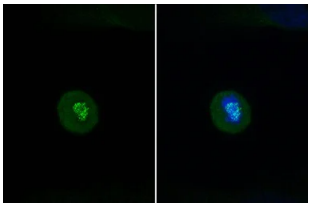


This image is courtesy of Scott Slattery and Mike Mancini

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

HEC1/HEC Immunocytochemistry/ Immunofluorescence staining using mouse Anti-HEC1/HEC antibody

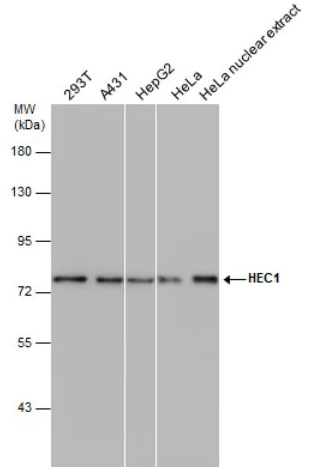
HeLa cells were stained with anti-HEC1/HEC (ab2613; in green) and DAPI (blue) in panel 1, and with anti-HEC1/HEC (green) and SH-CREST (red) to stain the centromeres in panel 2. Fix the cells 30 minutes on ice in 4% formaldehyde in PEM. Quench autofluorescence 2 x 5 min. with 1 mg/ml Na borohydride or 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.



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

HEC1/HEC Immunocytochemistry/ Immunofluorescence staining of HeLa cells using mouse Anti-HEC1/HEC antibody

Immunofluorescence analysis of HeLa cells fixed in 4% paraformaldehyde at RT for 15 min labelling Hec1 protein at kinetochore using ab3613 at a 1/500 dilution (Green). DAPI was used for nuclear staining (Blue).



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

HEC1/HEC Western blot staining using mouse Anti-HEC1/HEC antibody

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

Lane 1:
293T whole cell lysate at 30 µg

Lane 2:
A431 whole cell lysate at 30 µg

Lane 3:
HepG2 whole cell lysate at 30 µg

Lane 4:
HeLa whole cell lysate at 30 µg

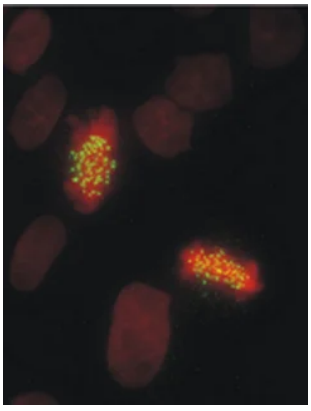
Lane 5:
HeLa nuclear extract at 30 µg

Secondary

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

Developed using the ECL technique.

Predicted band size: 74 kDa



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

HEC1/HEC Immunocytochemistry/ Immunofluorescence staining using mouse Anti-HEC1/HEC antibody

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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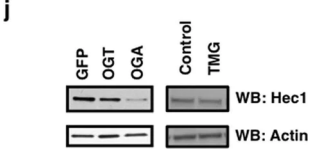


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Western blot - Anti-HEC1/HEC antibody [9G3] (ab3613)

HEC1/HEC Western blot staining using mouse Anti-HEC1/HEC antibody

HEC1/HEC western blot using anti-HEC1/HEC antibody [9G3] ab3613. Publication image and figure legend from Tan, E. P., Caro, S., et al., 2013, J Biol Chem, PubMed 23946484.

ab3613 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab3613 please see the product overview.

OGT or OGA gain of function disrupts spindle architecture. a, GFP, OGT, or OGA gain of function or OGA inhibitor Thiamet-G-treated HeLa cells were synchronized to M phase. Western blots were performed for O-GlcNAc, OGA, OGT, GFP, cyclin B, and actin. b–d, DNA (blue) and α-tubulin (green)

confocal imaging of M phase gain of function OGT/OGA cells, or cells treated with Thiamet-G. Midzone width and length (mean ± S.E., replicate number (n): nGFP = 195, nOGT = 193, nOGA = 198, nNT = 195, nTMG = 167, *, $p < 0.005$ between GFP/NT versus OGT/OGA or TMG) were quantified using ImageJ software as follows: first, the condensed chromatin area color was inverted in order to delineate the edge of the chromatin. Next, the chromatin was measured lengthwise, and the width was measured three times from different sections and averaged. Yellow lines are a representative measurement. e, multipolar spindles were quantified from confocal images of DNA (blue) and γ-tubulin (green) M phase synchronized gain of function OGT/OGA cells. f, DNA (blue) and γ-tubulin (green) were confocal imaged at M phase in OGT/OGA gain of function cells. Distance between each centrosome and the midzone was quantified (mean ± S.E., replicate number (n): nControl = 93, nOGT = 64, nOGA = 76, *, $p < 0.005$ between Control versus OGT/OGA). g, spindle angle measurement schematic in OGT/OGA gain of function cells. The perpendicular black lines originating from the centrosomes and the center of the spindle midzone were used to calculate spindle angle shown in yellow and plotted as a histogram. h, DNA (blue) and HEC1 (red) were confocal imaged at M phase in OGT/OGA gain of function cells. Average width of HEC1 staining was quantified using ImageJ (mean ± S.E., replicate number (n): nControl = 21, nOGT = 47, nOGA = 50, *, $p < 0.005$ between Control versus OGT/OGA). i, DNA (blue), α-tubulin (green), and HEC1 (red) were confocal imaged at M phase in TMG-treated cells. j, Western blot of HEC1 and actin in OGT/OGA gain of function and TMG-treated cells.

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