abcam

Product datasheet

Anti-Aurora B antibody ab2254

Overview

Product name Anti-Aurora B antibody

Description Rabbit polyclonal to Aurora B

Host species Rabbit

Tested applications Suitable for: ICC/IF, IHC-P, WB

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Hamster, Pig

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

General notesThe 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 pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

agent. If you would like information about the formulation of a specific lot, please contact our

scientific support team who will be happy to help.

Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

1

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab2254 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	★★★★★ (13)	Use a concentration of 0.5 - 1 µg/ml. Methanol fixation recommended.
IHC-P	★★★★ <u>(4)</u>	1/200.
WB	★★★★ ★ (14)	1/1000 - 1/2000. Detects a band of approximately 39 kDa (predicted molecular weight: 39 kDa).

Target

Function

May be directly involved in regulating the cleavage of polar spindle microtubules and is a key regulator for the onset of cytokinesis during mitosis. Component of the chromosomal passenger complex (CPC), a complex that acts as a key regulator of mitosis. The CPC complex has essential functions at the centromere in ensuring correct chromosome alignment and segregation and is required for chromatin-induced microtubule stabilization and spindle assembly. Phosphorylates 'Ser-10' and 'Ser-28' of histone H3 during mitosis. Required for kinetochore localization of BUB1 and SGOL1. Interacts with INCENP.

Tissue specificity

High level expression seen in the thymus. It is also expressed in the spleen, lung, testis, colon, placenta and fetal liver. Expressed during S and G2/M phase and expression is up-regulated in cancer cells during M phase.

Involvement in disease

Note=Disruptive regulation of expression is a possibile mechanism of the perturbation of chromosomal integrity in cancer cells through its dominant-negative effect on cytokinesis.

Sequence similarities

Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. Aurora subfamily.

Contains 1 protein kinase domain.

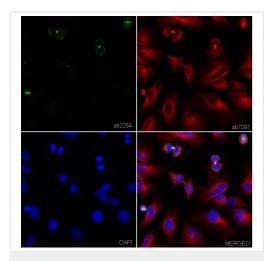
Post-translational modifications

Ubiquitinated by different BCR (BTB-CUL3-RBX1) E3 ubiquitin ligase complexes. Ubiquitinated by the BCR(KLHL9-KLHL13) E3 ubiquitin ligase complex, ubiquitination leads to removal from mitotic chromosomes and is required for cytokinesis. During anaphase, the BCR(KLHL21) E3 ubiquitin ligase complex recruits the CPC complex from chromosomes to the spindle midzone and mediates the ubiquitination of AURKB. Ubiquitination of AURKB by BCR(KLHL21) E3 ubiquitin ligase complex may not lead to its degradation by the proteasome.

Cellular localization

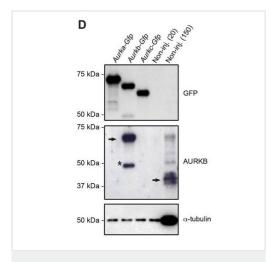
Nucleus. Chromosome. Chromosome > centromere. Cytoplasm > cytoskeleton > spindle. Localizes on chromosome arms and inner centromeres from prophase through metaphase and then transferring to the spindle midzone and midbody from anaphase through cytokinesis. Colocalized with gamma tubulin in the mid-body.

Images



Immunocytochemistry/ Immunofluorescence - Anti-Aurora B antibody (ab2254)

ab2254 stained in Hela cells. Cells were fixed with 4% paraformaldehyde (10min) at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% triton for 1h at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab2254 at 1 μ g/ml and ab7291 (Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control) at 1/1000 dilution overnight at +4°C. The secondary antibodies were ab150120 (pseudo-colored red) and ab150081 (colored green) used at 1 ug/ml for 1hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43 μ M for 1hour at room temperature.



Western blot - Anti-Aurora B antibody (ab2254)

Balboula and Schindler PLoS Genet. 2014 Feb 27;10(2):e1004194. doi:

10.1371/journal.pgen.1004194. eCollection 2014 Feb. Fig 1. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

AURKB is expressed in mouse oocytes.

(Panel D) 20 GV-intact oocytes were collected from CF1 mice and micro-injected with the indicated cRNA. Two hours after injection, the oocytes were matured to Met II *in vitro* (16 h). The total numbers of non-injected control oocytes (Non-inj.) are indicated in parenthesis. Total cellular lysates were probed with the indicated antibody. The panels are images of the same membrane that was stripped and re-probed. The arrows indicate the specific AURKB protein band, and the asterisk indicates a presumed degradation product of AURKB-GFP.

A **AURKB** DNA Merge GV Met I Met II

Immunocytochemistry/ Immunofluorescence - Anti-Aurora B antibody (ab2254)

Balboula and Schindler PLoS Genet. 2014 Feb 27;10(2):e1004194. doi: 10.1371/journal.pgen.1004194. eCollection 2014 Feb. Fig 1.

HCT 116 (Human colorectal carcinoma cell line) cells were examined by immunofluorescence confocal microscopy.

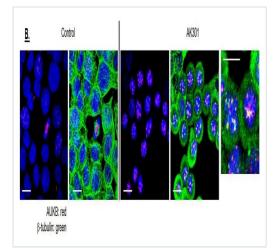
AURKB is expressed in mouse oocytes.

staining with an anti-AURKB antibody (ab2254).

(Panel A) GV-intact oocytes were collected from CF1 mice and matured in vitro for 8 h (Met I), or 16 h (Met II), prior to fixation and

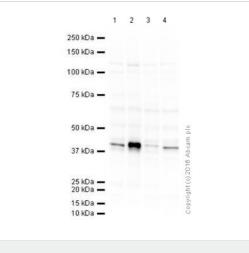
Cells were treated with 500 nM AK301 for 16 hours, and then processed for Aurora B (ab2254) and β-tubulin staining (**Panel B**). The color key and 20 µm bars are shown. Images of representative field is shown with a 20 µm bar. End-labeled DNA is shown in red and DAPI-stained DNA is blue.

Cells cultured on coverslips were fixed with 4% paraformaldehyde at room temperature or 100% ice cold methanol at 4°C and then permeabilized with 0.5% Triton X-100 in PBS. Cells were blocked in 5% serum (in PBS) and then incubated with primary antibody (in 5% serum) on shaker for 1 h at room temperature.



Immunocytochemistry/ Immunofluorescence - Anti-Aurora B antibody (ab2254)

Chopra et al PLoS One. 2016 Apr 20;11(4):e0153818. doi: 10.1371/journal.pone.0153818. eCollection 2016. Fig 6. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/



Western blot - Anti-Aurora B antibody (ab2254)

All lanes: Anti-Aurora B antibody (ab2254) at 1 µg/ml

Lane 1: HeLa cell lysate

Lane 2: HeLa nocodozole treated cell lysate

Lane 3 : NIH3T3 cell lysate
Lane 4 : PC12 cell lysate

Lysates/proteins at 10 µg per lane.

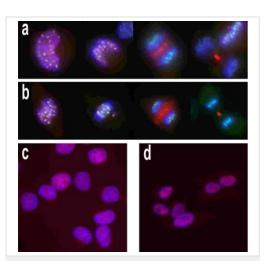
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

Performed under reducing conditions.

Predicted band size: 39 kDa

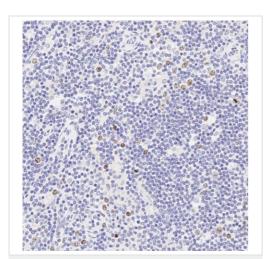
Blocked with 2% BSA.



Immunocytochemistry/ Immunofluorescence - Anti-Aurora B antibody (ab2254)

Immunofluorescence in human cells using Rabbit polyclonal to Aurora B (red), DAPI (blue) and CREST serum (binds to centromeres)(green).

- (a) HeLa cells transition from interphase (left) through mitosis
- (b) RPE-1 cells as in (a)
- (c) HeLa cells interphase
- (d) RPE-1 cells interphase



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Aurora B antibody (ab2254)

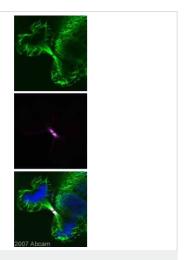
IHC image of Aurora B staining in Human Lymph node Hodgkins disease formalin fixed paraffin embedded tissue section*, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab2254, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

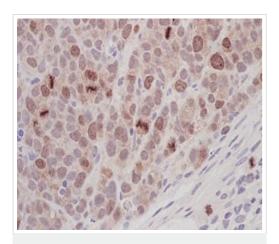
ab2254 staining human A431 (epithelial) cells by ICC/IF. The sample was fixed in paraformaldehyde and permeabilized by incubation with 0.1% Triton X100. 1% BSA was used as the blocking agent prior to a 1 hour incubation with the primary antibody, diluted 1/1000 with 1% BSA made up in PBS. An Alexa Fluor® 647 conjugated Donkey anti-Rabbit IgG (H+L) antibody was used as the secondary. Blocking and antibody incubation steps were carried out at room temperature.

In this set of images, the tubulin is stained green, Aurora B in pink and DNA in blue.



Immunocytochemistry/ Immunofluorescence - Anti-Aurora B antibody (ab2254)

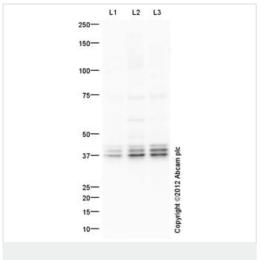
This image is courtesy of an Abreview from Lux Fatimathas.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Aurora B antibody (ab2254)

Rabbit polyclonal to Aurora B (ab2254) used to stain SW620 human tumour xenografts (in mouse).

The sections were microwave pretreated in citrate buffer (pH 6.0) for 5 mins high then 5 mins simmer (800W conventional microwave). Slides were then incubated for 1 hour with the Aurora B primary antibody diluted 1/200 in TBS, then visualised using DAB, after application of an appropriate secondary.



Western blot - Anti-Aurora B antibody (ab2254)

All lanes: Anti-Aurora B antibody (ab2254) at 1 µg/ml

Lane 1 : HeLa Whole Cell Lysate

Lane 2 : HeLa Nuclear Lysate

Lane 3: Jurkat Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) ($\underline{ab97051}$) at 1/10000

dilution

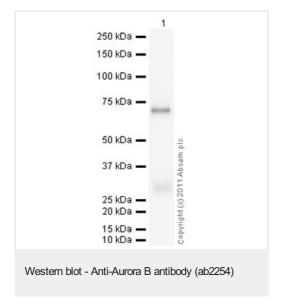
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 39 kDa **Observed band size:** 39 kDa

Additional bands at: 37 kDa (possible isoform)

Exposure time: 150 seconds



Anti-Aurora B antibody (ab2254) at 1/2000 dilution + Recombinant human Aurora B protein (ab51435) at 0.1 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 39 kDa

Exposure time: 30 seconds

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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