Antibody: Anti-Aurora A antibody [35C1] ab13824

**Description:** Mouse monoclonal [35C1] to Aurora A

**Host species:** Mouse

**Specificity:** The specificity of ab13824 was confirmed by showing that it binds in ELISA to recombinant Aurora A, it detects a 46kd band on HeLa cell extract and it binds to duplicated centrosomes and spindle poles in MCF7 cells. This antibody does not inhibit the kinase activity of Aurora A. This antibody has been successfully used to detect Aurora A in the following cell extracts: 293T, HMEC, T47D, MCF7, MDA-MB-468, SK-Br-3, S68 and HeLa.

**Tested applications:** Suitable for: WB, ELISA, ICC, ICC/IF, IP, IHC-Fr, IHC-P, Flow Cyt

**Species reactivity:** Reacts with: Mouse, Human

**Immunogen:** Recombinant full length his-tagged Aurora A protein (Human).

**Positive control:** Human HeLa and mouse M-ICc12 cell lysates for Western blotting and human 293 or mouse LLC1 cell lines for IF. In Flow Cytometry, this antibody gave a positive signal in HeLa cells.

**General notes:** This antibody clone is manufactured by Abcam. If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here.

**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:** Preservative: 0.09% Sodium Azide
Constituents: PBS, pH 7.4

**Purity:** IgG fraction

**Clonality:** Monoclonal

**Clone number:** 35C1

**Myeloma:** Sp2/0-Ag14

**Isotype:** IgG2b
Function
Contributes to the regulation of cell cycle progression. Required for normal mitosis. Associates with the centrosome and the spindle microtubules during mitosis and functions in centrosome maturation, spindle assembly, maintenance of spindle bipolarity, centrosome separation and mitotic checkpoint control. Phosphorylates numerous target proteins, including ARHGEF2, BRCA1, KIF2A, NDEL1, PARD3, PLK1 and BORA. Regulates KIF2A tubulin depolymerase activity (By similarity). Required for normal axon formation. Plays a role in microtubule remodeling during neurite extension. Important for microtubule formation and/or stabilization.

Tissue specificity
Highly expressed in testis and weakly in skeletal muscle, thymus and spleen. Also highly expressed in colon, ovarian, prostate, neuroblastoma, breast and cervical cancer cell lines.

Sequence similarities
Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. Aurora subfamily. Contains 1 protein kinase domain.

Post-translational modifications
Activated by phosphorylation at Thr-288; this brings about a change in the conformation of the activation segment. Phosphorylation at Thr-288 varies during the cell cycle and is highest during M phase. Autophosphorylated at Thr-288 upon TPX2 binding. Phosphorylated upon DNA damage, probably by ATM or ATR. Ubiquitinated by CHFR, leading to its degradation by the proteasome (By similarity). Ubiquitinated by the anaphase-promoting complex (APC), leading to its degradation by the proteasome.

Cellular localization
Cytoplasm > cytoskeleton > centrosome. Cytoplasm > cytoskeleton > spindle pole. Detected at the neurite hillock in developing neurons (By similarity). Localizes on centrosomes in interphase cells and at each spindle pole in mitosis.

Applications
Our Abpromise guarantee covers the use of ab13824 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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<tr>
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<th>Abreviews</th>
<th>Notes</th>
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<tbody>
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<td>Use a concentration of 1 µg/ml. Detects a band of approximately 46 kDa.</td>
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<td>Flow Cyt</td>
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<td>Use 2µg for 10^6 cells. ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.</td>
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Target

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All lanes: Anti-Aurora A antibody [35C1] (ab13824) at 5 µg/ml

Lane 1: Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate

Lane 2: HEK293 (Human embryonic kidney cell line) Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 46 kDa

Observed band size: 50 kDa

why is the actual band size different from the predicted?

Additional bands at: 125 kDa (possible non-specific binding), 55 kDa (possible non-specific binding)

Exposure time: 20 minutes

This blot was produced using 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200v for 50 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab13824 over night at 4°C. Antibody binding was detected using an anti-mouse antibody conjugated to HRP, and visualised using ECL development solution.
ab13824, at 1/2000 dilution, detecting Aurora A (green) in Hela Cells in conjunction with a Goat anti-mouse secondary antibody conjugated to Cy3®. Cells were fixed with methanol and counterstained with DAPI. Please refer to abreview for further details.

ICC/IF image of ab13824 stained HeLa cells. The cells were 100% Methanol fixed (5 min) and then incubated in 1%BSA / 10% normal Goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab13824, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 Goat anti-Mouse IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.
Overlay histogram showing HeLa cells stained with ab13824 (red line). The cells were fixed with 80% methanol (5 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. The cells were then incubated with the antibody (ab13824, 2µg/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 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.

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