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Datasheet for 600-401-677

AURORA KINASE B phospho T232 Antibody

Overview

Description:	Anti-Aurora B pT232 (RABBIT) Antibody - 600-401-677
Item No.:	600-401-677
Size:	100 µg
Applications:	ELISA, IHC, WB, IF, Multiplex
Reactivity:	Human, Monkey
Host Species:	Rabbit

Product Details

Background:	Aurora Kinase B (Aurora-B) is a Ser/Thr protein kinase member of the Aurora subfamily that may be directly involved in regulating the cleavage of polar spindle microtubules and is a key regulator for the onset of cytokinesis during mitosis. Aurora Kinase B is localized to the midzone of central spindle in late anaphase and concentrated into the midbody in telophase and cytokinesis and is colocalized with gamma tubulin in the mid-body. High levels of Aurora B expression are seen in the thymus, although it is also expressed in the spleen, lung, testis, colon, placenta and fetal liver. Aurora B is expressed during S and G2/M phase and expression is up-regulated in cancer cells during M phase. Anti-AUROA B pT232 Antibody is useful for researchers interested in gene expression, DNA damage, cytokinesis, and transcription activities.
Synonyms:	rabbit anti-Aurora B pT232 Antibody, Phospho Aurora B, AIK2 antibody, AIM1 antibody, ARK2 antibody, AurB antibody, AURKB antibody, Aurora 1 antibody, Aurora and Ipl1 like midbody associated protein 1 antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	AURKB
Reactivity:	Human, Monkey
PTM Specificity:	Phosphorylation

Immunogen Type:	Conjugated Peptide
Immunogen:	This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to an internal region surrounding T232 of Human Aurora Kinase B protein.
Purity/Specificity:	Anti-Phospho Aurora B pT232 affinity purified antibody is directed against the phosphorylated form of human Aurora Kinase B at the pT232 residue. The product was affinity purified from monospecific antiserum by immunoaffinity purification. Antiserum was first purified against the phosphorylated form of the immunizing peptide. The resultant affinity purified antibody was then cross-adsorbed against the non-phosphorylated form of the immunizing peptide. Reactivity occurs against human Aurora Kinase B pT232 protein and the antibody is specific for the phosphorylated form of the protein. Reactivity with non-phosphorylated human Aurora Kinase B is minimal by ELISA. No reaction is expected against Aurora Kinase A. However, 100% sequence homology as indicated by BLAST analysis is on record for this protein from human, mouse, rat, cow, pig, dog and chimpanzee. Cross reactivity with Aurora Kinase B from other sources is not known.
Relevant Links:	• UniProtKB - Q96GD4 • NCBI - 83776600 • GeneID - 9212 • SDS

Application Details

Tested Applications:	ELISA, IHC, WB
Suggested Applications:	IF, Multiplex (Based on references)
Application Note:	Phospho pT232 Aurora B antibody has been tested for use in ELISA, immunohistochemistry, and by western blot. See below for specific protocol. Expect a band approximately 39 kDa in size corresponding to Aurora Kinase B by western blotting in the appropriate cell lysate or extract. HeLa cell lysate can be used as a positive control.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:30,000
IF:	User Optimized
IHC:	User Optimized
WB:	1:250 - 1:2,000

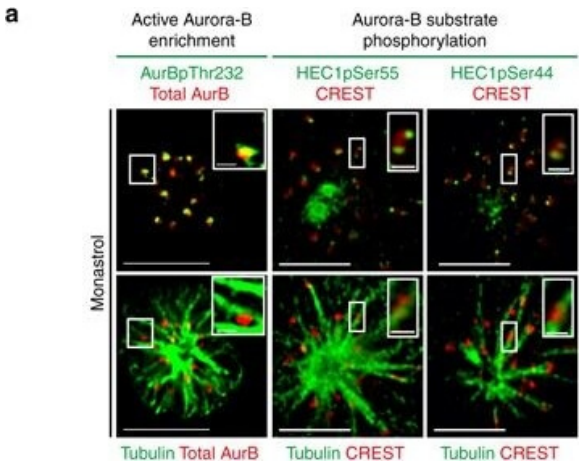
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	0.87 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

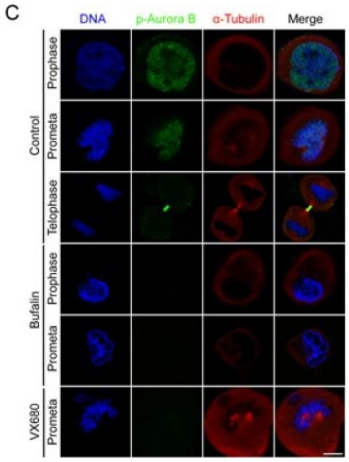
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store Phospho Specific Antibody at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images

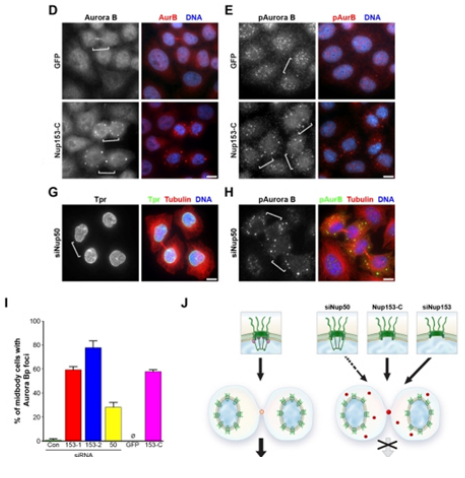


Immunocytochemistry
High Aurora-B activity promotes KT attachment to MT-walls. a Representative images show high Aurora-B activity on lateral kinetochores. Monastrol treated cells were immunostained with antibodies against Tubulin, Aurora-BpThr232 and total Aurora-B (AurB) (left panel) or CREST antisera and antibodies against Tubulin and either HEC1pSer55 (middle panel) or HEC1pSer44 (right panel). Cropped images show lateral-kinetochores. Scale: 5 µm in uncropped images; 1 µm in cropped images. b Graphs show higher average signal intensities of HEC1pSer55 (left) and HEC1pSer44 (right) in lateral compared to end-on kinetochores as assessed from at least nine randomly chosen kinetochores from cells in a. CREST signal intensities are used as internal controls. c Experimental regime: Cells transfected with plasmid vectors encoding Mis12-INCENP-GFP were exposed to Monastrol and MG132 with either ZM447439 or DMSO (solvent control), prior to immunostaining. d Images of cells expressing Mis12-INCENP-GFP treated as in c and immunostained with antibodies against Tubulin, SKAP and GFP. White arrowheads in cropped images show 'Lateral' kinetochore lacking SKAP (upper panel) and 'End-on' kinetochore enriched with SKAP (lower panel). Scale: 5 µm in uncropped and 2 µm in cropped images. Boxed areas in a and d correspond to cropped images. e Graph shows percentage of lateral, end-on and detached kinetochores in Mis12-INCENP-GFP expressing cells treated as in c. Each circle represents value from one cell. Black horizontal bar marks average values from three independent experimental repeats. '*' indicates statistically significant difference on the basis of P-values obtained using unpaired Student's t-test Figure provided by CiteAb. Source: Nat Commun, PMID: 28751710.

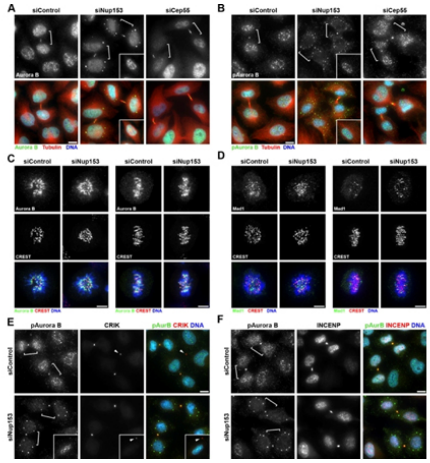


Immunocytochemistry

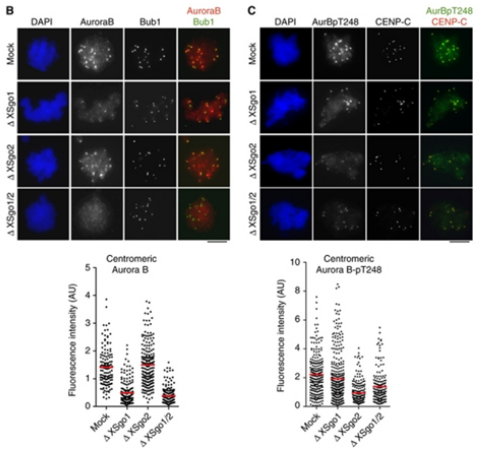
Bufalin prevents Aurora A recruitment to mitotic centrosomes and Aurora B recruitment to unattached kinetochores(A) HeLa cells were synchronized by a single thymidine treatment, released in the presence or absence of bufalin (100 nM) for 9 h, and stained for phospho-Aurora A (Green), α-tubulin (Red) and DNA (Blue). The scale bar represents 10 μm. (B) The phospho-Aurora A (Thr288) staining signals in (A) were normalized to the intensity in a same-size cytoplasmic region for at least five prometaphase cells per condition from three different experiments. ***$p < 0.001$, versus control prometaphase. Error bar represents SEM. (C) Thymidine-synchronized HeLa cells were treated with or without bufalin (100 nM) for 9 h and then stained for phospho-Aurora B (Green), α-tubulin (Red) and DNA (Blue). The scale bar represents 10 μm. (D) For quantification of the intensity of phospho-Aurora B (Thr232) in (C), more than 88 phospho-Aurora B (Thr232) staining signals from at least five prometaphase cells were analyzed each in control, bufalin (100 nM) and VX680 (0.5 μM) arrest. ***$p < 0.001$, versus control prometaphase. Error bar represents SEM. Figure provided by CiteAb. Source: Oncotarget, PMID: 29568394.



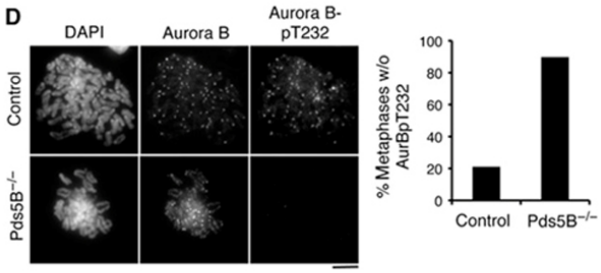
Immunofluorescence Microscopy
(D and E) Staining for both Aurora B (D) and pAurora B (p/n 600-401-677) (E) indicates that expression of Nup153-C–GFP leads to mislocalized and aberrantly active Aurora B in midbody-stage cells. (H) HeLa cells treated with Nup50-specific siRNA oligos display normal localization of Tpr (G) but display increased numbers of midbody-stage cells, many of which contain mislocalized pAurora B (H). (I) Quantification of the incidence of pAurora B foci in a midbody-stage cell after either siRNA treatment or fragment expression as indicated (n = 3). (J) Model for cross talk between nuclear pore assembly and the abscission checkpoint. Under normal conditions, resolution of cytokinesis ensues after proper nuclear pore assembly and Aurora B dephosphorylation (dashed red circle). pAurora B (solid red circles) is stabilized throughout the cytoplasm, including at the midbody, when assembly of the nuclear basket is disrupted, whether by a discrete change, such as the depletion of Nup50 (purple ovals), or a more potent disruption, as results from either interfering with Nup153 function (but not localization) by dominant interference (C terminus) or depleting Nup153. Activated Aurora B delays abscission, but the specific contributions of cytoplasmic versus midbody pools of Aurora B remain to be elucidated. (A, B, and D–H) Brackets indicate midbody-stage cells. Error bars indicate mean ± SD. Bars, 10 µm. Fig 5. PMID: 21098116



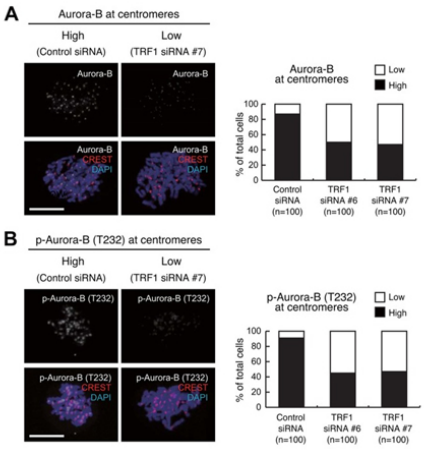
Immunofluorescence Microscopy
Aurora B is mislocalized and aberrantly active in midbody-stage cells depleted of Nup153. (A and B) siRNA-treated cells were stained for Aurora B (A) and pAurora B (p/n 600-401-677) (B). Note that Aurora B (and pAurora B) mislocalizes in cytoplasmic foci upon Nup153 depletion. Insets show normal Aurora B localization in interphase cells. (C and D) Localization of Aurora B (C) and the spindle checkpoint protein Mad1 (D) during prometaphase (left) and metaphase (right). CREST antiserum was used to indicate centromeres. (E) Detection of pAurora B and CRIK illustrates that mislocalized pAurora B foci are neither ectopic nor residual midbodies (arrowheads). Insets show a cell with what is likely a residual midbody. (F) Cells stained for pAurora B and INCENP. Brackets indicate midbody-stage cells. Bars: (A, B, E, and F) 10 µm; (C and D) 5 µm. Figure 3.
PMID: 21098116



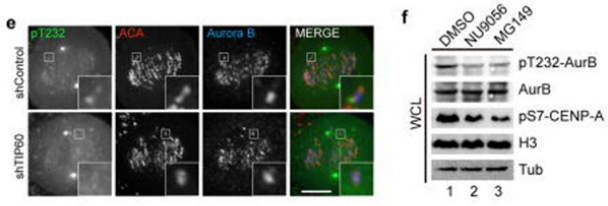
Immunofluorescence Microscopy
(B, C) Mitotic chromosomes were assembled in depleted extracts (as indicated) and immunostained with antibodies against Aurora B and Bub1 (B) or Aurora B-pT (p/n 600-401-677) and the centromeric protein CENP-C (C). DNA was counterstained with DAPI. The fluorescence intensity of Aurora B and Aurora B-pT248 stainings at individual centromeres is plotted. Data come from n>10 nuclei per condition from two experiments. Fig 5. PMID: 22274615



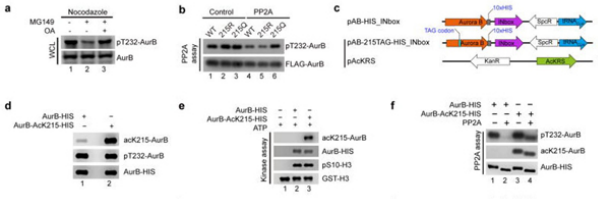
Immunofluorescence Microscopy
(D) Metaphase chromosomes from control and Pds5B−/− MEFs prepared by cytospin were labelled with Aurora B and phospho(T232) Aurora B (p/n 600-401-677). We scored the staining of 58 metaphases from 2 control clones and 45 metaphases from 2 Pds5B−/− clones. Scale bar, 10 µm. Fig 7. PMID: 24141881



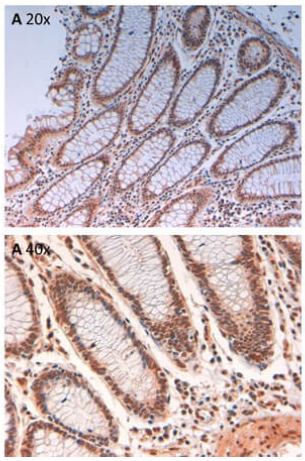
Immunofluorescence Microscopy
(A) (Left) Mitotic cells accumulated by exposure to nocodazole for 30 min were fixed and stained with Aurora-B (white) and CREST (red) antibodies and with DAPI (blue). Representative images of control and TRF1-depleted cells are shown. (Right) According to the signal intensity of Aurora-B that was colocalized with CREST (i.e., centromeres), cells were classified into two categories: high and low. We performed a randomized, double-blind study to classify the centromeric signals, which were of almost uniform intensity in each cell. Data were collected from at least three independent experiments. Bar, 10 µm.
(B) Centromeric localization of phospho-T232-Aurora-B [p/n 600-401-677] (white) was evaluated as described for panel A. Bar, 10 µm. Fig 4. PMID: 24752893



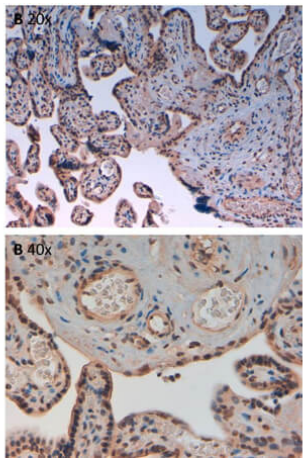
Immunofluorescence Microscopy
(e) HeLa cells expressing control shRNA or TIP60 shRNA were fixed and stained for the indicated antibody as illustrated.
(f) Mitotic HeLa cells were shaken off and treated with DMSO, NU9056 (20 µM), or MG149 (100 µM) for 1 h in the presence of MG132 before harvest. The cell lysates were separated by SDS-PAGE and blotted for the indicated antibodies to examine Aurora B kinase activity. See also Supplementary Fig. 9. Fig 2. PMID: 26829474



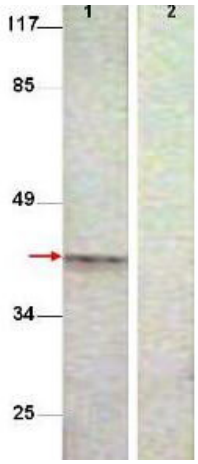
Western Blot
(a) Nocodazole arrested HeLa cells were treated with MG149 (100 µM) or MG149 plus OA (500 nM), and the Aurora B phosphorylation levels were assessed by Western blot.
(b) FLAG-tagged Aurora B was purified from lysates of OA (500 nM)-treated mitotic HeLa cells and incubated with PP2A phosphatase for 30 min. The reaction was then analyzed by Western blot.
(c) Diagram of plasmid combinations used to produce recombinant acK215-Aurora B or wild-type Aurora B in E. coli.
(d) Purified wild-type Aurora B and Aurora B bearing Lys215 acetylation were analyzed by Western blotting using acK215 and pT232 antibodies, respectively.
(e) Wild-type Aurora B or acK215-Aurora B was subjected to in vitro phosphorylation using GST-H3 as substrate.
(f) Recombinant Aurora B and Aurora B-acK215 were incubated with PP2A phosphatase for 2 h, and their phosphorylation levels were assessed by Western blot. Fig 4. PMID: 26829474



Immunohistochemistry
Immunohistochemistry of Rabbit Anti-AuroraB pT232 Antibody. Tissue: human intestine pH9 (A) at 20x and 40x. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: AuroraB pT232 antibody at 10 µg/mL for 1 h at RT. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10,000 for 45 min at RT. Localization: AuroraB pT232 is cytoplasmic. Staining: AuroraB pT232 as precipitated brown signal with hematoxylin purple nuclear counterstain.



Immunohistochemistry
Immunohistochemistry of Rabbit Anti-AuroraB pT232 Antibody. Tissue: human placenta pH9 (A) at 20x and 40x. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: AuroraB pT232 antibody at 10 µg/mL for 1 h at RT. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10,000 for 45 min at RT. Localization: AuroraB pT232 is cytoplasmic. Staining: AuroraB pT232 as precipitated brown signal with hematoxylin purple nuclear counterstain.



Western Blot
Western Blot shows detection of Aurora B protein at 39 kDa (predicted band size). All lanes : Aurora B (phospho T232) antibody diluted 1:500. Lane 1 : Extract from COS7 cells treated with Nocodazole (1ug/ml, 16 hrs). Lane 2 : Extract from COS7 cells treated with Nocodazole (1ug/ml, 16 hrs) and with the phosphopeptide immunogen.

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