

## Product Datasheet

# AURORA KINASE B phospho T232 Antibody (orb345466)

<b>Catalog Number</b>	orb345466
<b>Category</b>	Antibodies
<b>Description</b>	Aurora B (phospho-pT232) antibody
<b>Clonality</b>	Polyclonal
<b>Species/Host</b>	Rabbit
<b>Isotype</b>	IgG
<b>Conjugation</b>	Unconjugated
<b>Reactivity</b>	Human, Monkey
<b>Form/Appearance</b>	Liquid (sterile filtered)
<b>Concentration</b>	1.0 mg/mL
<b>Buffer/Preservatives</b>	Preservative: 0.01% (w/v) Sodium Azide. Stabilizer: None; Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

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<b>Purity</b>	Aurora B Phospho pT232 Antibody is 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.
<b>Immunogen</b>	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.
<b>UniProt ID</b>	<b>Q96GD4</b>
<b>Tested applications</b>	ELISA, IHC, WB
<b>Dilution range</b>	ELISA: 1:10,000 - 1:50,000, WB: 1:250 - 1:2,000
<b>Application notes</b>	Anti-Aurora B pT232 Antibody is affinity-purified antibody has been tested for use in ELISA, immunohistochemistry, and by western blot. 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.
<b>Antibody Type</b>	Primary Antibody
<b>Storage</b>	Store Phospho Specific Antibody at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.

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**Dry Ice Shipping**

**Please note: This product requires shipment on dry ice. A dry ice surcharge will apply.**

**Note**

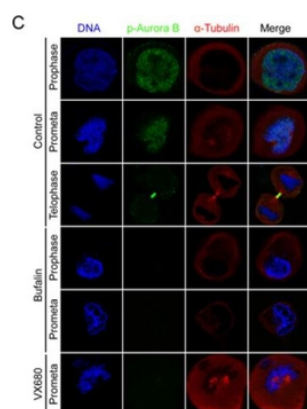
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**Expiration Date**

12 months from date of receipt.



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),  $\alpha$ -tubulin (Red) and DNA (Blue). The scale bar represents 10  $\mu$ m. (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),  $\alpha$ -tubulin (Red) and DNA (Blue). The scale bar represents 10  $\mu$ m. (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  $\mu$ m) arrest. \*\*\*p 0.001, versus control prometaphase. Error bar represents SEM.

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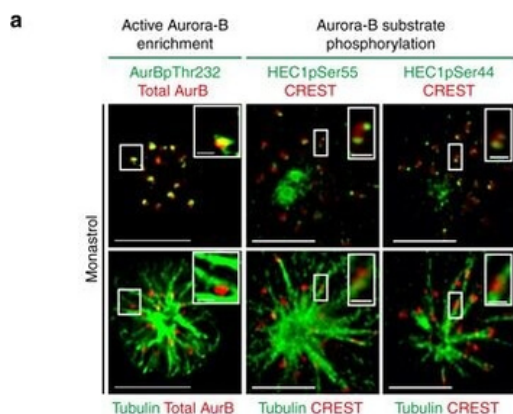
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High Aurora-B activity promotes KT attachment to MT-walls. a Representative images show high Aurora-B activity on lateral kinetochores. Monastrol treated cells were immunostained with antibodies against Tubulin, Aurora-BpThr232 and total Aurora-B (AurB) (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  $\mu$ m in uncropped images; 1  $\mu$ m 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  $\mu$ m in uncropped and 2  $\mu$ m 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

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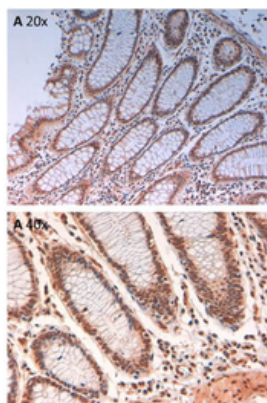
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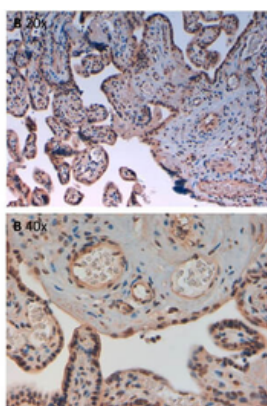
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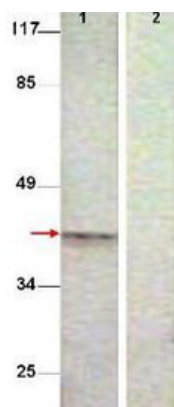
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Immunohistochemistry of Rabbit Anti-AuroraB pT232 Antibody. Tissue: human intestine pH9 (A) at 20x and 40x. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: AuroraB pT232 antibody at 10 µg/ml for 1 h at RT. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10000 for 45 min at RT. Localization: AuroraB pT232 is cytoplasmic. Staining: AuroraB pT232 as precipitated brown signal with hematoxylin purple nuclear counterstain.



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 1:10000 for 45 min at RT. Localization: AuroraB pT232 is cytoplasmic. Staining: AuroraB pT232 as precipitated brown signal with hematoxylin purple nuclear counterstain.



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 (1 µg/ml, 16 hrs). Lane 2 : Extract from COS7 cells treated with Nocodazole (1 µg/ml, 16 hrs) and with the phosphopeptide immunogen.

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