

Certificate of Analysis**Product:** Affinity Purified Anti-Human Aurora Kinase B pT232 (Rabbit)**Code:** 600-401-677**Lot #:** 20940**Size:** 100 µg**Physical State:** Liquid (sterile filtered)**Antibody Concentration:** 1.21 mg/ml (by UV absorbance at 280 nm)**Buffer:** 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2**Stabilizer:** None**Preservative:** 0.01% (w/v) Sodium Azide**Storage Conditions:** Store vial at -20° C prior to opening. Dilute only prior to immediate use. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening.**Background Information:** Aurora Kinase B (also known as Aurora and Ipl1-like midbody-associated protein 1, AIM-1, Aurora/IPL1-related kinase 2, Aurora-related kinase 2, STK-1, and 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.**Application Note(s):** This affinity-purified antibody has been tested for use in ELISA 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.

Recommended Dilutions:	ELISA	1:10,000 - 1:50,000
	WESTERN BLOT	1:250 - 1:2,000
	IF MICROSCOPY	User Optimized
	OTHER APPLICATIONS	User Optimized

Recommended Protocol(s): Western blotting.

1. Grow up 3X 10cm plates of MCF-7 cells in regular DMEM supplemented with 10% FBS.
2. Wash cells 2X with 1X PBS.
3. Lyse cells by adding 400 µl of Mammalian Protein Extraction Reagent (M-PER™, PIERCE) or PTY buffer (50mM HEPES pH7.5, 50mM NaCl, 5mM EDTA, 1% Triton X-100, 50mM NaF, 10mM Na₄P₂O₇, supplemented with protease inhibitors 1mM PMSF, 0.01mg/ml leupeptine, 0.01mg/ml aprotinin and phosphatase inhibitor 1mM Na₃VO₄). Let stand for 5-10 min.
4. Scrape the cells using a Cell Lifter (Fisher). Transfer the lysate to a 1.5 ml tube and centrifuge at maximum speed (~14,000rpm) for 10 min at +4°C to clear the lysate. From this point on, protein lysate must be maintained **on ice**.
5. Transfer the supernatant (lysate) to a clean centrifuge tube. Perform a protein determination. Aliquot the lysate in 100 µg units.
6. Use 50-100 µg for the detection of total level of Aurora B protein or 200-500 µg for phosphorylated form (pT232) Aurora B.
7. Add 10 µl of 10X SDS-loading buffer and boil for 3 to 5 min in a water bath.
8. Load the content of the tube onto a 10% SDS-PAGE gel and separate by electrophoresis.
9. Transfer to the PVDF membrane (Millipore) in Tris-Glycine Buffer with 15% Methanol. **DO NOT ADD SDS!**
10. Wash 2X in dH₂O.
11. Incubate with blocking solution (5% BSA, 0.1%Tween-20 in 1X TBS) for 1h.
12. Add primary Antibody for 1h. For total Aurora B use code #600-401-465 at a 1:1,000 dilution. For phospho T232 detection use code #600-401-677 at a 1:250 dilution. Gently rock antibody-membrane reaction +4° C overnight.
13. Wash 3X in 1X TBS with 0.1%Tween-20 for 10-15 min per wash.
14. Add secondary antibody HRP-Gt-a-RABBIT IgG (code #611-103-122) at a 1:10,000 dilution for 1h.
15. Wash 3X in 1XTBS with 0.1%Tween-20.
16. Develop by ECL-Plus (Amersham) or West-Pico (PIERCE).

Purity and Specificity: : This 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 Link(s): Swiss Prot: [Q96GD4](#) NCBI Link [NP_004208](#).

Protein Sequence: Human Aurora B Kinase, 344 aa, predicted MW 39.3kDa

1	maqkensypw	pygrqtapsg	lstlpqrivr	kepvtpsavl	lmsrsnvqpt	aapgqkvmen
61	ssgtpdiltr	hftiddfeig	rplgkkgfgn	vylarekksh	fivalkvlfk	sqiekegveh
121	qlrreiieqa	hlhpnirli	ynyfydrri	ylileyaprg	elykelqksc	tfdeqrtati
181	meeladalm	chgkkvihrd	ikpenlllg	kgelkiadfg	ws vhapslrr	ktm cgltidyl
241	ppemiegrmh	nekvdlwcig	vlycyellvgn	ppfesashne	tyrrivkvdl	kfpasvptga
301	qdliskllrh	npserlplaq	vsahpwwran	srrvlppsal	qsva	

Immunogen: This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding aa 223-234 of Human Aurora Kinase B protein.

General References:

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- Gruneberg,U., Neef,R., Honda,R., Nigg,E.A. and Barr,F.A. (2004) Relocation of Aurora B from centromeres to the central spindle at the metaphase to anaphase transition requires MKlp2. *J. Cell Biol.* **166** (2), 167-172.
- Kimura,M., Uchida,C., Takano,Y., Kitagawa,M. and Okano,Y. (2004) Cell cycle-dependent regulation of the human aurora B promoter. *Biochem. Biophys. Res. Commun.* **316** (3), 930-936.
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- Araki,K., Nozaki,K., Ueba,T., Tatsuka,M. and Hashimoto,N. (2004) High expression of Aurora-B/Aurora and lpl-like midbody-associated protein (AIM-1) in astrocytomas. *J. Neurooncol.* **67** (1-2), 53-64.
- Wheatley,S.P., Henzing,A.J., Dodson,H., Khaled,W. and Earnshaw,W.C. (2004) Aurora-B phosphorylation in vitro identifies a residue of survivin that is essential for its localization and binding to inner centromere protein (INCENP) in vivo. *J. Biol. Chem.* **279** (7), 5655-5660.
- Honda,R., Korner,R. and Nigg,E.A. (2003) Exploring the functional interactions between Aurora B, INCENP, and survivin in mitosis. *Mol. Biol. Cell* **14** (8), 3325-3341.
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Related Products:

- [#600-401-465](#) Affinity Purified Anti-Human Aurora B pT232 (Rabbit)
- [#600-401-677](#) Affinity Purified Anti-Human Aurora B pT232 (Rabbit)
- [#611-703-127](#) Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
- [#611-132-122](#) IRDye800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
- [#MB-070](#) Blocking Buffer for Fluorescent Western Blotting
- [#KIA-003](#) **MaxTag**TM Anti-Rabbit IgG Kit for Immunoblotting

USDA Certification: All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation.

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.