

Antibodies to the Aurora Kinase Family

Aurora kinases are serine/threonine kinases (STKs) that are essential for cell proliferation. Chromosomal segregation during mitosis as well as meiosis is regulated by kinases and phosphatases. The Aurora kinases associate with microtubules during chromosome movement and segregation. Aurora kinase B localizes to microtubules near kinetochores, specifically to the specialized microtubules called K-fibers, and Aurora kinase A localizes to centrosomes. Aurora kinases have generated significant interest in the cancer research field due to their elevated expression profiles in many human cancers. Three enzymes have been identified in mammalian cells to date:

Aurora kinase A (AURKA, STK6)

Zhou et al. (1998, PMID 9771714) found that Aurora kinase A is involved in the induction of centrosome duplication-distribution abnormalities and aneuploidy in mammalian cells. Centrosomes appear to maintain genomic stability through the establishment of bipolar spindles during cell division, ensuring equal segregation of replicated chromosomes to two daughter cells. Deregulated duplication and distribution of centrosomes are implicated in chromosome segregation abnormalities, leading to aneuploidy seen in many cancer cell types. In addition, amplification of STK6 is demonstrated in approximately 12 % of primary breast tumors, as well as in breast, ovarian, colon, prostate, neuroblastoma and cervical cancer cell lines. High expression of STK6 mRNA is detected in tumor cell lines without evidence of gene amplification. Ectopic expression of STK6 in mouse NIH 3T3 cells leads to the appearance of abnormal centrosome

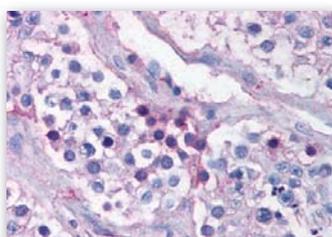


Fig. 1: Human testis (formalin-fixed, paraffin-embedded; FFPE) stained with Acris Aurora kinase A antibody Cat.-No. AP07481PU-N, 1:50, followed by biotinylated secondary antibody, alkaline phosphatase-streptavidin and chromogen

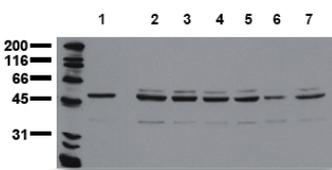


Fig. 2: Detection of endogenous Aurora kinase A: Whole cell extracts of vanadate treated tumor cells (20,000 cells per lane) were applied to SDS-PAGE and transferred to a PVDF membrane. The immunoblot was probed with Acris Aurora kinase A antibody clone 7F12 Cat.-No. AM20207PU-N (0.5 µg/ml) for 1h at RT and developed by ECL: lane 1: A431; lane 2: SW480; lane 3: SW620; lane 4: HT29; lane 5: MCF-7; lane 6: MDA-MB231; lane 7: T47D

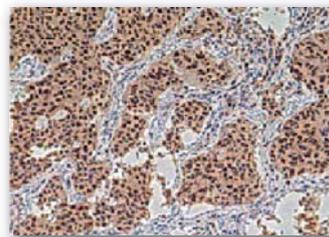


Fig. 3: Immunoperoxidase staining of pancreatic cancer tissue (FFPE) with Acris Aurora kinase A antibody Cat.-No. SP2058P

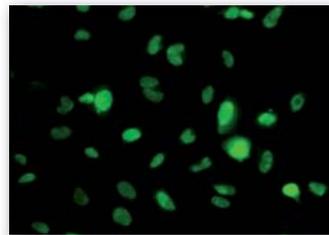


Fig. 4: Immunofluorescence of monoclonal Abnova antibody to Aurora kinase A Cat.-No. H00006790-M01 on HeLa cell [antibody concentration 30 µg/ml]

number (amplification) and transformation *in vitro*. Finally, overexpression of STK6 in near-diploid human breast epithelial cells reveals similar centrosome abnormality, as well as induction of aneuploidy. Aurora kinase A is a key regulatory component of the p53 pathway, and overexpression of Aurora kinase A leads to increased degradation of p53, causing downregulation of checkpoint-response pathways and facilitating oncogenic transformation of cells. These findings suggested that AURKA is a critical kinase-encoding gene, whose overexpression leads to centrosome amplification, chromosomal instability and transformation in mammalian cells.

Aurora Kinase B (AURKB, STK12)

This protein 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 the central

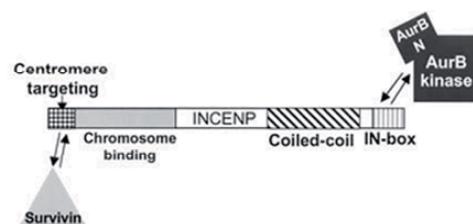


Fig. 5: Model of Aurora kinase B, inner centromere protein (INCENP) and survivin interactions and regulation. Survivin binds the N-terminus of INCENP, whereas the N terminus of Aurora kinase B binds the C-terminus of INCENP

spindle in late anaphase, concentrated into the midbody in telophase and cytokinesis, and is colocalized with gamma tubulin in the midbody. High levels of Aurora kinase B expression are seen in the thymus, although it is also expressed in the spleen, lung, testis, colon, placenta and fetal liver. Aurora kinase B is expressed during S and G₂/M phase, and expression is up-regulated in cancer cells during M phase.

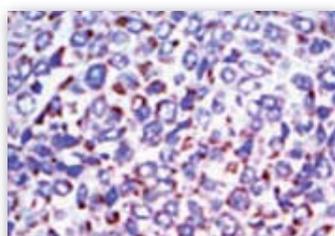


Fig. 6: Human hepatocarcinoma tissue (FFPE) reacted with Acris Aurora kinase B antibody Cat.-No. AP13522PU-N using peroxidase-conjugated secondary antibody followed by AEC staining

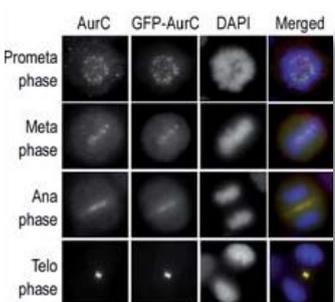


Fig. 7: In the immunofluorescence experiment, staining of HeLa cells expressing GFP-Aurora kinase C is performed at different cellular mitotic stages with the Acris Aurora kinase C antibody Cat.-No. AP13519PU-N as primary antibody (column A), GFP fluorescence (column B), DAPI nuclear staining (column C), and anti-Aurora kinase C merged to DAPI staining (column D). Data is kindly provided by Drs. K. Sasai and S. Sen, University of Texas, MD Anderson Cancer Center (Houston, TX)

Aurora Kinase C (AURKC, STK13)

This protein acts as a chromosomal passenger protein and forms complexes with Aurora kinase B and inner centromere protein (INCENP). Aurora kinase C phosphorylates histone H₃ *in vitro*. INCENP binds and activates AURKC *in vivo* and *in vitro*, and AURKC co-expressed with INCENP elicits histone H₃ phosphorylation of interphase cells. Using an *in vitro* kinase assay, Yan et al. (2005, PMID 15938719) found that AURKC-SV phosphorylates a test substrate protein, and its activity depends on Thr179. During cell mitosis, AURKC-SV associates with chromosomes in prophase and metaphase, and then transferred to the central spindle midzone and the cortex where the contracting ring forms during the transition from anaphase to telophase. It remains in the midbody during cytokinesis.

Selected Antibody Panel to Aurora Kinase Subfamily

Acris Antibodies offers a collection of polyclonal and monoclonal antibodies to members of the Aurora kinase family. These antibodies are thoroughly evaluated and suitable for Western blotting and immunohistochemistry methods, and also for other immunological applications, such as immunocytochemistry and immunoprecipitation. This collection is extended by Abnova antibodies distributed by Acris Antibodies within Europe and the USA.

Selected Antibody Panel to Aurora Kinase Family Members

Name	Property	Host/Isotype	Clone	Reactivity	Application	Catalog-No.
Aurora kinase A	pThr288	Rabbit IgG	-	Hu	P, Wb	AP07481PU-N
	-	Goat	-	Hu	E, P, WB	AP07516PU-N
	pThr288	Rabbit	-	Hu, Ms	P	AP08036PU-S
	N-term	Rabbit	-	Hu	E, P, WB	AP13523PU-N
	N-term	Rabbit	-	Hu	E, P, WB	AP14756PU-N
	-	Rabbit	-	Hu	IP, P	SP2058P
	N-term	Mouse IgG1	7F12	Can, Hu, Ms, Rt	WB	AM20207PU-N
	-	Mouse IgG2b	35C1	Hu, Ms	IF, IP, WB	SM2084P
-	Mouse IgG2a	5F8	Hu	E, IF, WB	H00006790-M01	
Aurora kinase A/B	-	Rabbit	-	Hu, Ms, Rt	WB	SP7343P
	C-term	Mouse IgG1	5F11	Hu	F, IF, WB	AM20208PU-N
Aurora kinase B	-	Rabbit	-	Hu	E, IF, P, WB	AP07400PU-N
	-	Rabbit	-	Hu	E, IF, P, WB	AP07530PU-N
	N-term	Rabbit	-	Hu	E, P, WB	AP13522PU-N
	-	Sheep	-	Hu	WB	BP4524
	-	Mouse IgG2b	AT2B1	Hu	E, IF	AM09372PU-N
	-	Mouse IgG1	6A6	Hu	E, WB	H00009212-M01
Aurora kinase C	N-term	Rabbit	-	Hu	E, IF, WB	AP13518PU-N
	N-term	Rabbit	-	Hu	E, IF, WB	AP13519PU-N
	N-term	Rabbit	-	Hu	E, IF, WB	AP13520PU-N

Can: Canine, Hu: Human, Ms: Mouse, Rt: Rat

E: ELISA, IP: Immunoprecipitation, F: Flow cytometry, IF: Immunofluorescence, IP: Immunoprecipitation, P: Immunohistochemistry on formalin-fixed, paraffin-embedded tissue sections, WB: Western blot

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