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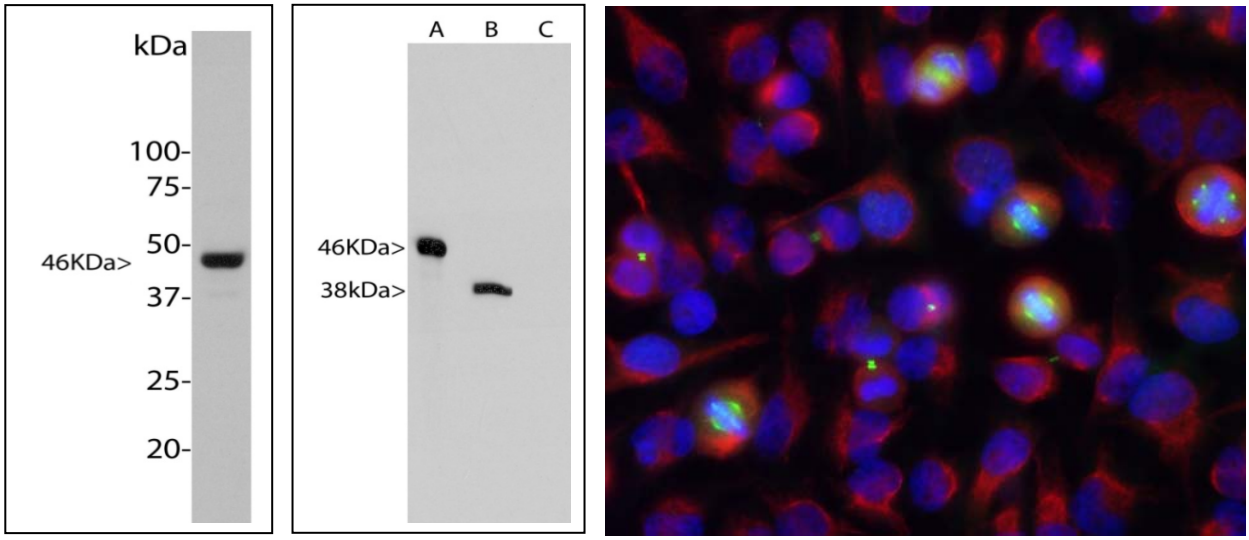
Catalogue# MCA-3H1: Mouse Monoclonal Antibody to Aurora A and B Kinases

The Immunogen: Aurora kinases are family of serine/threonine protein kinases that play a key role in mitosis progression. The first Aurora kinase was discovered in Drosophila. Because mutations of this kinase cause monopolar spindles, it was given the name "Aurora", reminiscent of the North Pole (1). Mammalians contain at least three aurora kinases: Aurora A, Aurora B, and Aurora C. Aurora kinases comprise mainly two domains: a regulatory domain in the NH2 terminus and a catalytic domain in the COOH terminus. The regulatory domain is diverse largely, whereas the catalytic domain shares more than 70% homology among aurora A, aurora B, and aurora C (2). Aurora A is required for centrosome duplication, entry into mitosis, formation of bipolar spindle and mitotic checkpoint (3). Aurora B is a chromosomal passenger protein and essential for chromosome condensation, kinetochore functions, spindle checkpoint activation and cytokinesis completion (4). Aurora C is largely expressed in testis and mostly involved in spermatogenesis (5). Aurora A is first associated with centrosomes and then with spindle microtubules whereas aurora B localizes to the spinal midzone and finally accumulates at the midbody. MCA-3H1 was raised against full length recombinant human aurora A expressed in and purified from E. coli. The antibody was tested for binding to expressed human aurora A, B, C and shown to react with aurora A and aurora B (see below). The HGNC names for aurora A and B are AURKA and AURKB.



Left: Western analysis of MCA-3H1 in HeLa cells. Blot of HeLa cells that were treated with 100 ng/mL nocodazole for 18 hours was probed with MCA-3H1. Nocodazole is microtubule inhibitor which induces cells G2/M phase and induces aurora A expression. The MCA-3H1 monoclonal binds strongly to a band at about 46 kDa: aurora A and weakly to a band at 38 kDa: aurora B. **Middle**: Blot of expressed human aurora A, B and C was probed with MCA-3H1. This antibody reacted with aurora A at 46kDa and aurora B at 38 kDa. **Right:** HeLa cell cultures stained with MCA-3H1 antibody (green). Aurora A localizes in spindle poles and mitotic spindles at anaphase, while aurora B localizes in midzone at metaphase and concentrates on the midbody between the two daughter cells during telephase. Counterstained is our chicken polyclonal antibody against Vimentin: **CPCA-Vim** (red). Blue is the DNA Dapi stain.

Antibody characteristics: MCA-3H1 is a mouse IgG1 class antibody with a k light chain. MCA-3H1 recognizes aurora A kinase specifically both in western blots and in immunocytochemical experiments. On blots, MCA-3H1 reveals a prominent 46 kDa band and a weak 38 kDa band, on cells in tissue cultures, MCA-3H1 reveals strong staining in spindle poles and midbodies.

Suggestions for use: The antibody is protein G purified from tissue culture supernatant and is diluted in phosphate buffered saline at 1 mg/mL. The preparation contains 10 mM sodium azide preservative (Link to http://www.encorbio.com/MSDS/azide.htm for a material safety data sheet (MSDS). The antibody solution can be used at dilutions 1:500-1:1,000 for immunofluorescence. For western blots try at 1:1,000.

Storage Instructions: Shipped on ice. Please store at 4°C for regular uses. For long term storage, please leave frozen at -20°C and avoid freeze/thaw cycles.

Limitations: This product is for research use only and is not approved for use in humans or in clinical diagnosis

References:

1: Glover DM, Leibowitz MH, McLean DA, Parry H. Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles. Cell 81:95-105 (1995).

2: Hochegger H, Hegarat N, Pereira-Leal JB. Aurora at the pole and equator: overlapping functions of Aurora kinases in the mitotic spindle. Open Biol. Mar 20;3(3):120185 (2013)

3: Barr AR, Gergely F. Aurora-A: the maker and breaker of spindle poles. J Cell Sci.120:2987-96 (2007). Review.

4: Andrew PD, Knatko E, Moore WJ, Swedlow JR. Mitotic mechanics: the auroras come into view. Curr Opin Cell Biol.15(6):672-83 (2003). Review.

5: Tang CJ, Lin CY, Tang TK. Dynamic localization and functional implications of Aurora-C kinase during male mouse meiosis. Dev Biol. Dev Biol. 290(2):398-410 (2006).