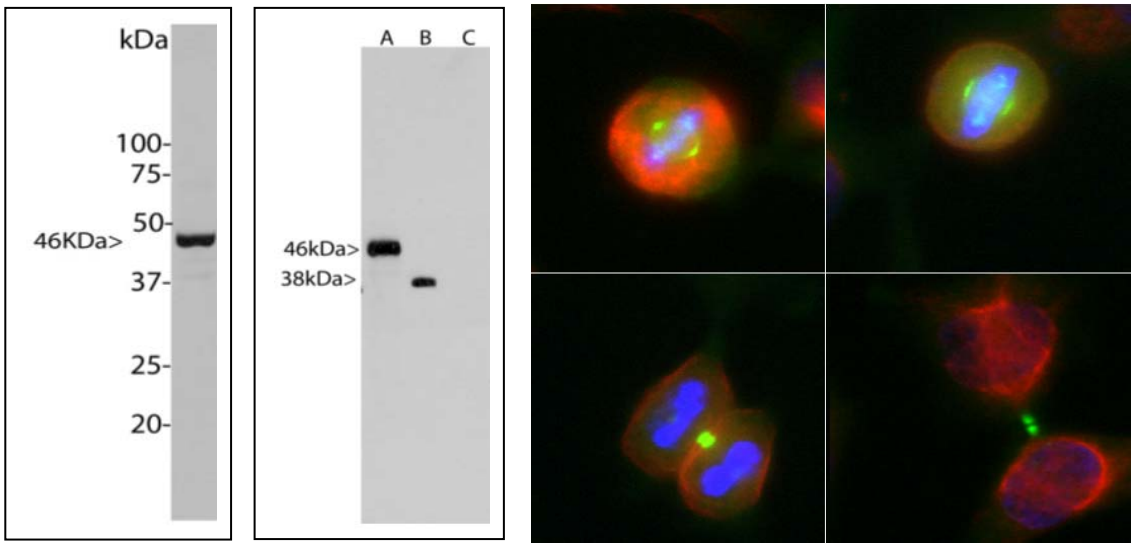
4949 SW 41st Blvd.
Suites 40 & 50
Gainesville, FL 32608
Tel: (352) 372 7022
Fax: (352) 372 7066
admin@encorbio.com

Catalogue# MCA-5A12: Mouse Monoclonal Antibody to Aurora A and B Kinases

The Immunogen: Aurora proteins are a family of serine/threonine protein kinases that play a key role in the regulation of cell division. The first Aurora kinase was discovered in *Drosophila* (1). Mutations of this kinase cause monopolar spindles surrounded by kinase, and the appearance of this was reminiscent of the Aurora borealis at the poles of the earth (1). Mammals contain three Aurora kinases named Aurora A, Aurora B, and Aurora C. All three contain a regulatory domain in the N terminus which is quite different between the three molecules followed by a catalytic serine/threonine kinase domain which is almost identical between the three. To download a sequence alignment of the 3 human Aurora proteins go to http://www.encorbio.com/Alignments/Aurora%20alignment.pdf. As a consequence antibodies raised against one Aurora family member frequently cross-react with other family members. There is a short C-terminal peptide which is also variable between the three molecules (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 heavily expressed in testis and is there involved in spermatogenesis, but is also expressed in many cell lines and has been less well studied to date (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-5A12 was raised against full length recombinant human Aurora A expressed in and purified from *E. coli*. The antibody was tested for binding to recombinant human Aurora A, B and C and shown to react with both Aurora A and B, but not C (see Blot image). The HGNC names for Aurora A, B and C are AURKA, AURKB and AURKC. Since this antibody cross-reacted between Aurora A and B, we now have in progress monoclonal antibodies specific for only one Aurora gene product which will be posted on our website soon.



Figures: Left: Western blot analysis of MCA-5A12. Blot of HeLa cells treated with 100 ng/mL nocodazole for 18 hours was probed with MCA-5A12 (left). Nocodazole is microtubule inhibitor which induces cells G2/M phase and induces aurora A expression. The MCA-5A12 monoclonal binds strongly to aurora A at about 46 kDa and wealy to aurora B at 38 kDa. **Middle:** Blot of human recombinant proteins aurora A, B and C was probed with MCA-5A12 (right). This antibody reacted with aurora A at 46kDa and aurora B at 38 kDa, but not to aurora C. **Right:** HeLa cells were stained with MCA-5A12 antibody (green). The antibody stains spindle poles and mitotic spindles at anaphase (top 2 panels) and concentrates on the midbody between the two daughter cells during telophase (bottom 2 panels). It is therefore a useful marker of dividing cells. Cells were counterstained with our chicken polyclonal antibody against Vimentin **CPCA-Vim** in red. Blue is a DNA Dapi stain.

Antibody characteristics: MCA-5A12 is a mouse IgG1 class antibody with a k light chain. MCA-5A12 recognizes aurora A/B kinase specifically both in western blots and in immunocytochemical experiments. On blots, MCA-5A12 reveals a prominent 46 kDa band and a weak 38 kDa band, on cells in tissue cultures, MCA-5A12 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 dilutions1:1,000-1:2,000 for immunofluorescence and western blots.

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).