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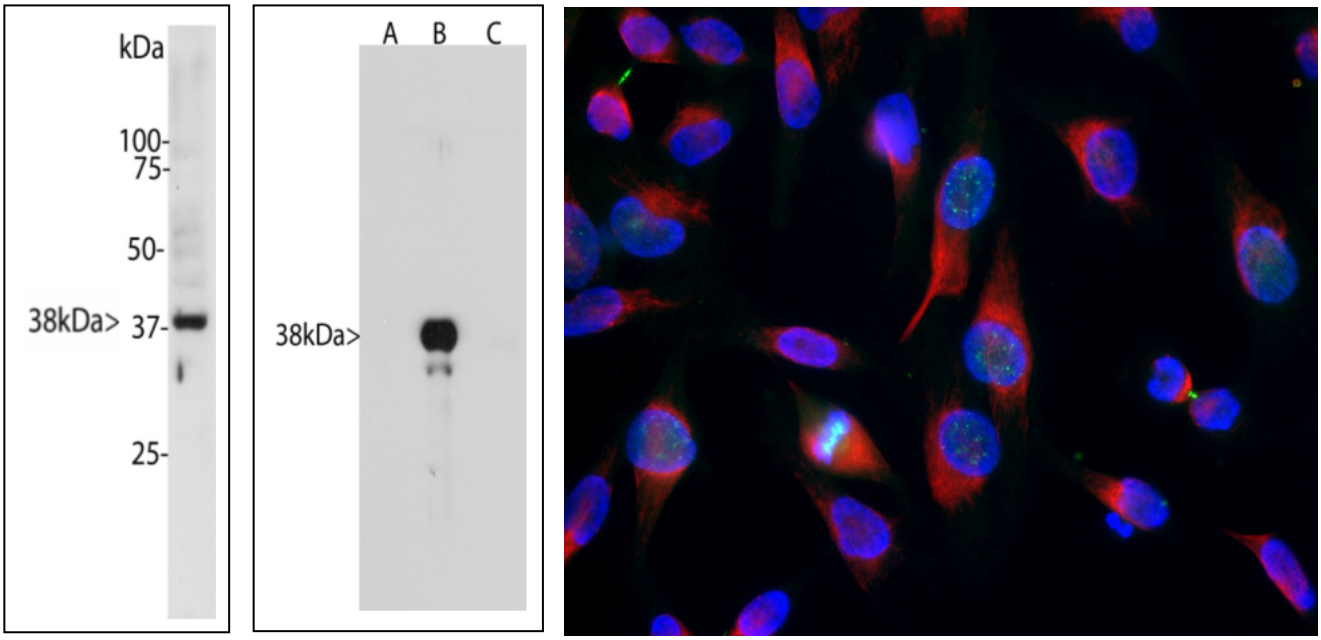
Catalogue# MCA-6G2: Mouse Monoclonal Antibody to Aurora B Kinase

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). Mammalian genomes encode 3 Aurora kinases named Aurora A, Aurora B, and Aurora C. All 3 contain a regulatory domain at the N terminus which is quite different between the molecules followed by a catalytic serine/threonine kinase domain which is almost identical between them. To download a sequence alignment of the 3 human Aurora proteins go to http://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 involved in spermatogenesis, but is also expressed in many cell lines and cancer cells 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-6G2 was raised against full length recombinant human Aurora B expressed in and purified from *E. coli*. The antibody was tested for binding to expressed human Aurora A, B and C and shown to react with only aurora B, not aurora A and C (see Blot image). The HGNC name for Aurora B is AURKB.



Left: Western blot analysis of MCA-6G2 in HeLa cells. Blot of HeLa cells treated with 100ng/ml nocodazole for 18 hours was probed with MCA-6G2. Nocodazole is a microtubule polymerization inhibitor which induces cells to halt at the G2/M phase and also induces Aurora B expression. The MCA-6G2 monoclonal binds strongly to aurora B at 38 kDa. **Middle:** Blots of recombinant human Aurora A, B and C were probed with MCA-6G2. This antibody binds specifically to Aurora B. This is also consistent with the immunocytochemical staining of midzones and midbodies seen on HeLa cells. **Right:** HeLa cell cultures were stained with MCA-6G2 antibody (green). Localization of aurora B is cell cycle phase dependent. First, Aurora B staining was seen in chromosome arm in cells in prophase (green small dots). During anaphase, Aurora B localized in midzone and then concentrated in midbodies between the two daughter cells during telophase. It is therefore a useful

marker of midbodies and dividing cells. Cells were counterstained with our chicken polyclonal antibody to Vimentin **CPCA-Vim** in red. Blue is the DNA Dapi stain.

Antibody characteristics: MCA-6G2 is a mouse IgG1 class antibody with a κ light chain. MCA-6G2 recognizes aurora B kinases in western blots and in immunocytochemical experiments.

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:

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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).