NEUROMICS

Aurora A/B kinase Data Sheet

Catalog Number:	MO22159	**Host:**	Mouse
Product Type:	Monoclonal IgG1 Affinity Purified Antibody	**Species Reactivity:**	Human, horse, cow, pig, chicken, rat, mouse
Immunogen Sequence:	Full length recombinant human protein aurora A expressed in and purified from E. coli HGNC name for this protein is AURKA, AURKB	**Format:**	Liquid, 100 ul aliquot Concentration: 1 mg/ml
Applications:	Immunofluorescence/Immunocytochemistry: 1:500-1:1,000 Immunohistochemistry: 1:500-1:1,000 Western Blot: 1:1,000		
Storage:	Dilutions listed as a recommendation. Optimal dilution should be determined by investigator. Antibody can also be aliquoted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Avoid repeated freeze-thaw cycles.		

Application Notes

Description/Data:

Aurora proteins are a family of serine/threonine kinases crucial for cell cycle control. Mutations of this kinase caused the formation of monopolar spindles surrounded by kinase, and the appearance of this was reminiscent of the Aurora borealis at the poles of the earth. Mammals express three closely related Aurora kinases named Aurora A, Aurora B, and Aurora C.

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. As a consequence antibodies raised against one Aurora family member frequently cross-react with other family members. Since there is a short C-terminal peptide which is also variable between the three molecules. 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. MO22159 is an excellent reagent for the visualization of mid bodies, centrosomes and spindles in dividing cells. The antibody was tested for binding to expressed human Aurora A, B and C and shown to react with both Aurora A and B, but not C (see Blot image).

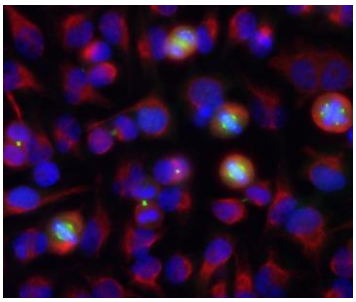

Image: HeLa cell cultures were stained with MO22159 antibody (green). The antibody stains spindle poles and mitotic spindles at anaphase and concentrates on the midbody between the two daughter cells during telophase. It is therefore a useful marker of dividing cells. Cells were counterstained with our chicken polyclonal antibody to Vimentin CH22108 red. Blue is a DNA stain.

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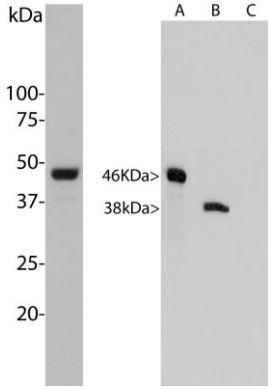

Left: Western blot analysis of MO22159 in HeLa cells. Blot of HeLa cells treated with 100ng/ml nocodazole for 18 hours was probed with MO22159. Nocodazole is a microtubule depolymerizing agent which induces cells to halt at the G2/M phase and also induces Aurora A expression. The MO22159 monoclonal binds strongly to a band at about 46 kDa, which is Aurora A and also shows binding to a band at 38 kDa. **Right:** Blots of recombinant human Aurora A, B and C were probed with MCA-3H1, which binds to both Aurora A and B. We conclude that the 38 kDa band seen in the HeLa extract is Aurora B. This is also consisted with the immunocytochemical staining of both centrosomes and midbodies seen on HeLa cells.

Immunostaining of cells in tissue culture:

The purpose of fixation is denaturing the components of cells enough so that they stay on the dish and can be bound by antibodies, hopefully without destroying cellular morphology. Fixatives such as formalin, paraformaldehyde and glutaraldehyde chemically cross-link proteins, by binding to amino acid side chains, generally the most chemically reactive ones like amines (Lysine, Arginine, Glutamine and Asparagine). This chemical modification can also have the consequence of blocking antibody binding sites. Substances such as acetone and methanol are not true fixatives but are denaturants, which precipitate proteins without covalently modifying them. We routinely use a combination of mild formalin fixation followed by cold methanol for neurons, mixed neuron/glial cultures and most of the widely used human and rodent cell lines. The formalin preserves the cellular morphology quite well, while the methanol further denatures the proteins of the cells and helps keep what is left of the cell adherent to the dish. For soluble proteins it may be necessary to miss the methanol step, but in this case you have to be very careful with the washing steps, as the cells tend to wash off the dish. Certain antibodies may be very sensitive to formalin fixation, so you may have to experiment a little, perhaps missing out that step. The following procedure works for antibodies to most cytoskeletal and signaling molecules. This procedure is good for cells in 6 well tissue culture plates or in 35mm tissue culture dishes. These are just big enough that you can look from above with a typical immunofluorescence microscope through a glass coverslip. This allows you to see the specimens very well and take very high quality pictures. (However note that it's a pain to changes lenses on the microscope if you use the 6 well dishes, since you have to rack the lens right the way up to do this, and you have to take out the two neighboring lenses in the turret since they will hit the other wells of the dish! It's less of a problem with 35mm dishes but still a pain. No procedure is perfect.…).

1. Draw of culture medium with aspirator and add 1 mL of 3.7 % formalin in PBS solution to the dish. (make up from 10 mLs Fisher 37% formalin plus 90mls PBS, the Fisher formalin contains 37% formaldehyde plus about 1% methanol which may be relevant sometimes). Let sit at room temp for 1 minute. (can add 0.1% Tween 20 to PBS used here and all subsequent steps to reduce background; probably best not to do this first time round though as it may extract your antigen or help wash your cells off the dish).

2. Take off the formalin/PBS and add 1ml of cold methanol (-20°C, kept in well-sealed bottle in fridge). Let sit for no more than 1 minute.

3. Take off methanol and add 1ml of PBS, not letting the specimen dry out. To block nonspecific antibody binding can add ~10 µL (=1%) of goat serum (Sigma), and can incubate for 30 minutes. Can then add antibody reagents. Typically, 100 µL of hybridoma tissue culture supernatent or 1ml of mouse ascites fluid or crude serum. Incubate for 1 hour at room temp. (or can go at 37°C for 30 minutes to 1 hour, or can do 4°C overnight, exact time not too critical). Can do very gentle shaking for well adherent cell lines (3T3, Hek293 etc.).

4. Remove primary antibody and replace with 1 mL of PBS. Let sit for 5-10 minutes, replace PBS and repeat twice, to give three washes in PBS.

5. Add 0.5 µLs of secondary antibody. These are fluorescently labeled Goat anti mouse or rabbit antibodies and are conjugated to ALEXA dyes and were originally marketed by Molecular Probes (Eugene Oregon, the ALEXA dyes are sulphonated rhodamine compounds and are much more stable to UV than FITC, TRITC, Texas red etc. Molecular Probes was bought by Invitrogen, which now markets these reagents). Typically make 1:2,000 dilutions of these secondaries in PBS plus 1% goat serum, BSA or non fat milk carrier. Incubate for 1 hour at room temp. (or can go at 37°C for 30 minutes to 1 hour, or can do 4°C overnight). Can do gentle shaking for well adherent cell lines (3T3, HEK293 etc.).

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6. Remove secondary antibody and replace with 1 ml of PBS. Let sit for 5-10 minutes, replace PBS and repeat twice, to give three washes in PBS.

7. Drop on one drop of Fisher mounting medium onto dish and apply 22 mm square coverslip. View in the microscope

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