Ki67

#Cat: NB-26-00509 **Size: 100UG**

Product Information

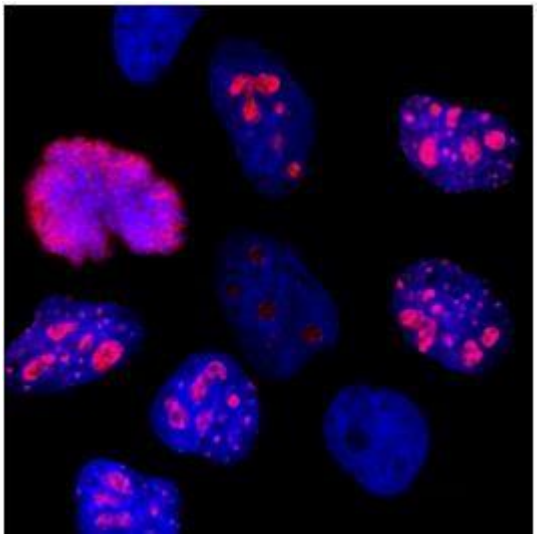
Code	NB-26-00509-100UG
Description/Data	The Ki67 proteins were first discovered in an attempt to generate cancer specific monoclonal antibodies. A monoclonal antibody which bound to structures in the nuclei of dividing but not quiescent cells wasproduced and shown to bind two very large proteins of molecular weight 345kDa and 395kDa. The two proteins were derived from alternate transcripts of a single gene. The presence of Ki67 proteins, detected with an appropriate antibody, is an indicator of cell proliferation and the level of Ki67 expression is one of the most reliablebiomarkers of proliferative status of cancer cells..
Product Type	Monoclonal
Host	Mouse
Species Reactivity	Human
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Immunogen Sequence	Recombinant construct, amino acids 317-630 of the human protein
Applications	Western Blot: 1:1,000-5,000 Immunofluorescence/Immunocytochemistry: 1:2,000-5,000. Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.

Immunostaining of cells In tissue culture:

The purpose of fixation is denaturing the components of cells enough so that they stayon 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 mostchemically 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 washoff 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 canlook 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 usethe 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-sealedbottle 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 ~10µL (=1%) of goat serum (Sigma), and can incubate for 30 minutes. Can then add antibody reagents. Typically, 100µL 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.).
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!



Immunofluorescent analysis of HeLa cells stained with mouse mAb to Ki67, MO22178, dilution 1:2,000 (red). The blue isDAPI staining of nuclear DNA.

For reference only

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