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|----------------------------|--|----------------------------|---|
| Catalog Number: | MO22151 | Host: | Mouse |
| Product Type: | Monoclonal IgG2b Affinity Purified Antibody | Species Reactivity: | human, cow, pig, mouse, rat and other mammals. |
| Immunogen Sequence: | Full length recombinant human SF3B4 expressed in and purified from <i>E. coli</i> | Format: | Liquid, 100 ul aliquot Concentration: 1 mg/ml |
| | HGNC name for this protein is SF3B4 | | |
| Applications: | Immunofluorescence/Immunocytochemistry: 1:1,000 Immunohistochemistry: 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:

Splicing factor SF3B4, also known as SAP49, is a ubiquitously expressed splicing factor found in the nuclei of eukaryotic cells, although it migrates into the cytoplasm of dividing cells. It was originally identified by as the protein most efficiently UV cross-linked to the A, B and C spliceosomal complexes (1).

The protein contains two of the highly conserved [RRM](#) type RNA recognition motifs, each corresponding to a small ~70 amino acid structure, consisting of 4 beta strands and two alpha-helices. Proteins containing these proteins are believed to have a role in the regulation of mRNA splicing.

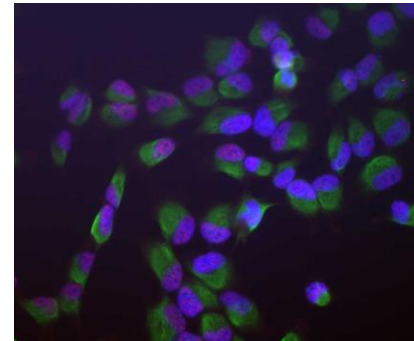
The protein runs on SDS-PAGE gels at an apparent molecular weight of 49kDa. This protein is known as splicing factor 3b, subunit 4, 49kDa SAP49, spliceosome-associated protein (U2 snRNP), Hsh49 and MGC108282. Antibodies to this protein are good markers of nuclei.

49kDa >



Right Image: Human HeLa cells stained with MO22151 (red), chicken antibody to vimentin ([CH22108](#)) (green) and DNA (blue, stained with DAPI). The MCA-3A1 antibody reveals strong granular nuclear staining which is a little different from the DNA stain and presumably reflects spliceosomal complexes. The vimentin antibody stains the cytoplasmic intermediate filament network of the HeLa cells.

Left Image: Blots of HeLa cell crude extract stained with MO22151. SF3B4 runs with an apparent SDS-PAGE molecular weight 49 kDa



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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/glia 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 change 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 off 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 supernatant 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

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