



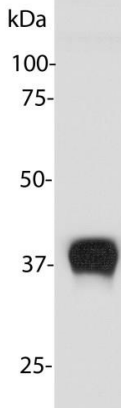
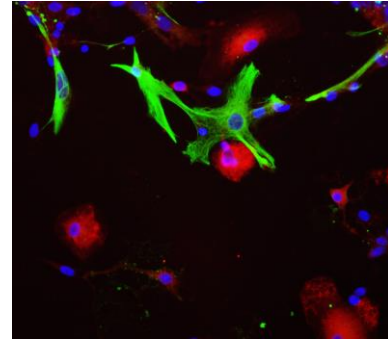
Catalog Number:	MO22156	Host:	Mouse
Product Type:	Monoclonal IgG1 Affinity Purified Antibody	Species Reactivity:	Human, horse, cow, pig, chicken, rat, mouse
Immunogen Sequence:	Recombinant human full-length aldolase C HGNC name for this protein is ALDOC	Format:	Liquid, 100 ul aliquot Concentration: 1 mg/ml
Applications:	Immunofluorescence/Immunocytochemistry: 1:1,000-5,00 Immunohistochemistry: 1:1,000-5,000 Western Blot: 1: 10,000		
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:

Aldolase A is generally considered to be a muscle enzyme. Northern analysis of cultured cells suggests that it is present in both neurons and glia. Aldolase C shares 81% amino acid identity with aldolase A and 70% identity with aldolase B.

Earlier studies using isozyme-specific antibodies report its location in gray matter astrocytes and cells of the pia mater. In situ hybridization of mouse central nervous system using isozyme-specific probes revealed that aldolase A and C are expressed in complementary cell types: aldolase A mRNA is found in neurons; aldolase C message is detected in astrocytes, some cells of the pia mater, and Purkinje cells. Aldolase C can in some situations be used as an astrocyte marker. However Purkinje cells of the cerebellum contain high levels of the enzyme, so the enzyme is not totally astrocyte specific.



Right Image: View of mixed neuron/glia cultures stained with MO22156 (red) and our chicken antibody to GFAP antibody (CH22102, green). MO22156 antibody reveals strong cytoplasmic staining in astrocytes. Blue is a DNA stain. Aldolase stains the astrocytes cell body and processes, whereas GFAP labels the intermediate filament of the cytoskeleton in subset of astrocytes.

Left Image: Blots of rat brain lysate blotted with MO22156. The MO22156 monoclonal binds strongly and cleanly to a band at about 40 kDa. We now know that this antibody binds to an epitope shared by the closely related Aldolase A, B and C, recognizing all three on western blots.

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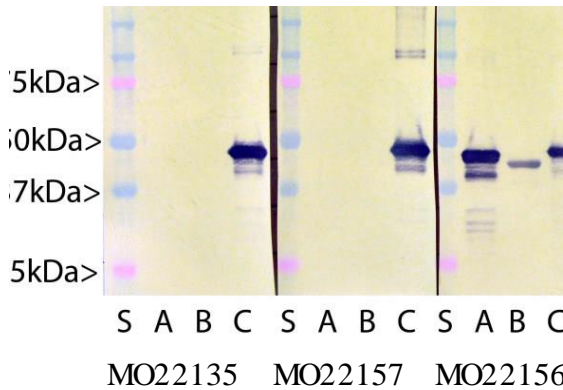


Image: Blots of recombinant human Aldolase A, B and C with MO22156 as indicated showing binding to all three gene products. Lane labelled S show molecular weight standards, while lanes A, B and C contain recombinant full length His-tagged human Aldolase A, B and C respectively. The epitope for MO22156 is within the core of the three Aldolase molecules which are less conserved than the N and C-termini where our Aldolase C specific antibodies bind. The specificity of our other two Aldolase antibodies is shown in the indicated parts of the image. One antibody binds to the N-terminal peptide MO22135 and one to the C-terminal peptide MO22157

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

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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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