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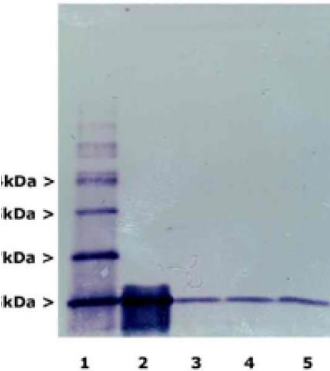
<b>Catalog Number:</b>	MO22152	<b>Host:</b>	Mouse
<b>Product Type:</b>	Monoclonal IgG1 Affinity Purified Antibody	<b>Species Reactivity:</b>	Human, horse, cow, pig, chicken, rat, mouse, Drosophila, C. elegans
<b>Immunogen Sequence:</b>	Purified ubiquitin conjugated with glutaraldehyde to KLH  HGNC name for this protein is UBB, UBC	<b>Format:</b>	Liquid, 100 ul aliquot Concentration: 1 mg/ml
<b>Applications:</b>	Immunofluorescence/Immunocytochemistry: 1:1,000 Immunohistochemistry: 1:1,000 Western Blot: 1:1,000		
<b>Storage:</b>	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.		

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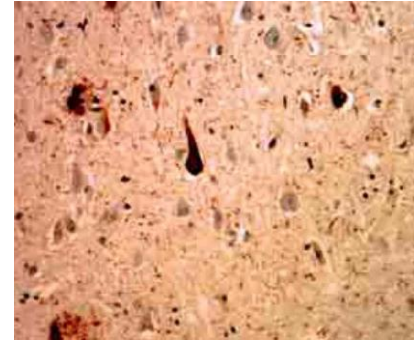
### Application Notes

#### Description/Data:

Ubiquitin is a highly conserved globular 76 amino acid protein of about 8.5 kDa molecular weight. It has an important role in the targeting of proteins for proteolytic degradation. Proteins to be degraded are covalently coupled to the C-terminus of ubiquitin by means of ubiquitin ligases. The ubiquitin itself is frequently also ubiquitinated, producing a polyubiquitin chain. The polyubiquitinated complex is then recognized by a complex of degradative enzymes which together form the proteasome. Interestingly, ubiquitin also becomes covalently bonded to many types of pathological inclusions seen in serious human disease states which appear to be resistant to normal



degradation, so that ubiquitin antibodies are very useful for studies of these inclusions. For example the neurofibrillary tangles and paired helical filaments diagnostic of Alzheimer's disease, the Lewy bodies seen in Parkinson's disease, and Pick bodies found in Pick's disease are all heavily ubiquitinated and can all be readily visualized with ubiquitin antibodies of appropriate specificity.



Right Image: MO22152 staining of cerebral cortex of an Alzheimer patient. Neurofibrillary tangles and dystrophic neurites associated with senile plaques stain strongly with this antibody. In the center is a typical neurofibrillary tangle containing neuron

Left Image: Blots of mono and polyubiquitin (lane 1), monoubiquitin only (2), and 100 µg total wet weight of cerebellum, cortex and brain stem respectively (lane 3-5). Material was run out on 20% SDS-PAGE and transferred electrophoretically to PVDF

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MO22152 was raised against purified ubiquitin conjugated with glutaraldehyde to keyhole limpet hemocyanin. This antibody can be used to study ubiquitinated inclusions seen in Alzheimer's and other kinds of disease; such as the Lewy bodies of Parkinson's disease and the Pick bodies in Pick's disease in formalin fixed tissues. It can be used on mildly fixed histological sections of human brain, and paraffin embedded material.

### **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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