



Catalog Number:	MO22116	Host:	Mouse
Product Type:	Mouse IgG _{2b} Mouse Monoclonal Antibody with a k light chain	Species Reactivity:	Human, Mouse, Rat, Cow
Immunogen Sequence:	High molecular MAP protein preparation derived from bovine brain.	Format:	100 ul. Purified tissue culture supernatant. Concentration of 1mg/ml in phosphate buffered saline preparation containing 10 mM sodium azide as a preservative
Applications:	Immunofluorescence: 1:1,000 Western Blot: 1:10,000		

Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.

Storage: Antibody can also be aliquotted 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

Immunostaining Cell Cultures

1. Draw of culture medium with aspirator and add 1 ml of 3.7 % formalin in PBS solution to the dish. (make up from 10mls 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 ~10ml (=1%) of goat serum (Sigma), and can incubate for 30 minutes. Can then add antibody reagents. Typically 100ml 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 mls of secondary antibody. These are fluorescently labeled Goat anti mouse antibodies and are conjugated to ALEXA dyes and are from Molecular probes (Eugene Oregon, the ALEXA dyes are sulphonated rhodamine compounds and are much more stable to UV than FITC, TRITC, Texas red etc.). 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 22mm square coverslip. View in the microscope!

Immunostaining Tissue

Solutions

PBS - sodium phosphate-buffered (100 mM; pH 7.2) isotonic (0.9% NaCl, w/v) saline Antibody dilution buffer (PBS with 0.1% non-ionic detergent, such as Triton X-100 or Tween-20) fluorescein anti-fading reagent -- Make up a 2 mg/ml phenylene diamine solution in PBS (phenylene diamine requires extensive vortexing to put it into solution). Once the

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phenylene diamine is completely dissolved, add an equal volume of glycerol and mix. This reagent will last about a week at -20°C. Discard this reagent when it starts to turn dark brown.

Other Reagents

Fluorescein-labeled goat anti-mouse IgG.

1. Prepare your tissue sections or cultured cells as you normally would. Wash your sections or cells for 1 min with PBS at room temperature.
2. Incubate your sections or cells with your chicken primary antibodies (diluted in "antibody dilution buffer") for at least 1 hour at room temperature. The concentration of your antibody may be anywhere from 1:50-1:150 depending on the titre of the antibody and the concentration of your antigen.
3. Wash your sections or cells over a 10 minute period at room temperature (with two changes of PBS).
4. Incubate your sections or cells with fluorescein-labeled goat anti-mouse IgG (1:500 dilution in "antibody dilution buffer" for 1 hour at room temperature. Be sure to keep these slides or culture dishes in subdued light (e.g., in a drawer) to avoid bleaching of the fluorescein dye.
5. Repeat step #4
6. Add a drop of "fluorescence anti-fading reagent" ([i-BRITE Plus](#)) to your sections or cells. Place a coverslip over the section. If you want to reduce messiness, you may also seal the coverslip by painting the edges with nail polish.
7. Store the slides or culture dishes in the refrigerator (in the dark).

Western Blotting

1. Run gel as usual. Take gel out of electrophoresis apparatus. Cut into segments as required; Part of gel can be stained directly in Coomassie brilliant blue R-250 (2.5 g Coomassie Brilliant Blue R-250, 450 mls methanol, 100 mls glacial acetic acid, water to 1 liter). Part to be used for electroblotting is put into tap water on shaker, after first having marked it unambiguously to identify top/bottom, left and right etc.
2. Leave in water on shaker for 5 minutes. This step can be substituted by washing the gel in electro-transfer buffer (see below) for 5 minutes.
3. We use a semidry blotter, which we have found to be quicker, more economical and easier than fully submerged blotting methods. We cut Whatman 3M filter papers to the size of our gels, and place three of these onto the semi dry blotter. These are then wet with transfer buffer (we routinely use 3.03 g Tris base, 14.4 g Glycine, 10% Methanol per liter). The gel is put onto the filters and a prewetted nitrocellulose filter is put on top of the gel. Alternately put a PVDF membrane on top; if you are using PVDF remember it is essential to prewet the PVDF in 100% methanol. Great care should be taken to ensure that no air bubbles are anywhere in this stack of membranes. Then three more wetted Whatman 3M filters should be placed on top of the pile, again taking great care not to have any bubbles in pile. Put the top onto the apparatus and screw it down. Proteins in transfer buffer are negative in charge mostly due to residual SDS and they therefore move from -ve to +ve pole. So the +ve electrode is above the nitrocellulose and the -ve side is below the gel.
4. Run for 30 minutes to 1 hour at ~100mA. The most reliable way of doing this is to use a powerful power supply 200-500mA and put it on constant voltage, with a setting of 5 to 10 Volts. Low molecular weight proteins (20kDa or less) will transfer in 30 minutes at 5 Volts, while higher molecular weight (150kDa or more) transfer in 60 minutes at 10 Volts.
5. After running disassemble the apparatus and remove nitrocellulose filter. Stain this for 5 minutes on shaker in Ponceau reagent (0.25% Ponceau S in 40% methanol and 15% acetic acid). Destain with regular SDS-PAGE gel destain solution (7.5% methanol, 10% acetic acid). If you transferred efficiently, the proteins can be seen as pale pink bands. This tells you whether the transfer was O.K. or not and also exactly where the bands are. You can photograph, photocopy or mark the position of the bands directly with a pencil. If you can't see any bands at this stage, it's probably smart to try to optimize steps 3 and 4. The gel may be discarded or may be stained as usual in coomassie, to see how much protein is left behind.
6. After Ponceau staining put the nitrocellulose filter into blocking solution, such as 1% bovine serum albumin (BSA) or 1% Carnation non fat milk (NFM), for 20 minutes to 1 hr at RT or 37°C. Since the NFM works just as well as BSA but is much cheaper, there is really no good reason to use BSA. Ponceau staining will fade to become completely invisible. Carry on with antibody incubations etc.

Antibody Incubations:

1. Put in antibody solutions. Volume should be enough to cover blot and allow it to float freely when you agitate. In initial experiments, antibody concentration should generally be about 1:100 - 1:1,000 for ascites, CL350 tissue culture supernatant or antiserum, undiluted to 1:10 for monoclonal supernatant, and about 1-10µg/ml for a pure IgG. If dilution brings antibody concentration to less than 50 µg/ml, add some BSA or NFM to act as carrier protein (e.g. make BSA or NFM concentration 1mg/ml). Incubate for at least 1 hour with shaking (can be room temperature or at 37°C, can also do overnight at 4°C).
2. Wash membranes in TBS (10mM Tris, 154mM NaCl, pH=7.5 plus 0.1% Tween 20) for 3 times at least five minutes each time with extensive agitation.
3. Incubate in second antibody (peroxidase-conjugate, phosphatase conjugate or radioactive). Add BSA or NFM carrier as before if necessary. Incubate for at least one hour at room temperature or 37°C can also do overnight at 4°C with shaking as before.
4. Wash membranes in TBS (10mM Tris, 154mM NaCl, pH=7.5 plus 0.1% Tween 20) for 3 times at least five minutes each time with extensive agitation.

A. Alkaline Phosphatase Blot System

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1. Incubate in alkaline phosphatase conjugated antibody against the primary antibody (e.g. Goat anti-mouse, rabbit or chicken; buy from Sigma or some other trusted source). Typical concentration is 1:1,000 in TBS (10mM Tris/HCl, 154mM NaCl, pH=7.5). Add a small amount of BSA or NFM to act as carrier. Incubate for 1 hour at room temperature (or 37°C) with shaking.
2. Wash in TBS three times 5 minutes each. (N.B. the alkaline phosphatase enzyme is inhibited by EDTA, which chelates zinc and magnesium, and by phosphate, which inhibits forward reaction. Make sure therefore you use TBS which is EDTA and phosphate free- Don't make up developer in PBS!)
3. Put into developer. Buffer is 100mM Tris/HCl, 100mM NaCl, 5mM MgCl₂ pH=9.5. To 10ml of this add 33µl BCIP-T (5-bromo-4-chloro-3-indolyl phosphate, p-toluidine salt, make up 50mg/ml in water or Dimethyl formamide; in water makes a yellow suspension) and 33µl of NBT (Nitro Blue Tetrazolium, also 50mg/ml in water). Can store these solutions at -20°C. Can buy this solution made up already from Sigma. Reaction product is purple, and appears in a few minutes; can incubate for up to an hour if the signal is weak. Watch development of reaction and stop with water. Some of background disappears on drying.

B. Horse Radish Peroxidase Staining

After washing of blots in TBS or PBS (must not have azide in wash buffer! This inhibits the peroxidase enzyme) add reaction mixture. This is; 20 mls 0.1M Tris/HCl pH=7.2 (Vecta stain buffer). 200 µl NiCl (80 mg/ml), 6 µl 30% hydrogen peroxide, 1ml of 5mgs/ml diaminobenzidine. (Wear gloves, DAB is carcinogenic). Alternate protocol; Make 20 mls ammonium acetate buffer (50mM, pH=5.0). Add 1 ml of 10mg/ml Diaminobenzidine, 40µl 30% hydrogen peroxide. Brown reaction product is seen in 1-10 minutes, not quite so nice as above method.

C. Chemiluminescence Staining

Chemiluminescence has an advantage of perhaps an order of magnitude greater sensitivity than the dye based methods above. In addition, several films may be exposed from a single blot, giving an advantage in interpretation of weak and strong signals on the same membrane. However it requires a darkroom to perform and is more expensive in reagents. Reagents are generally bought in a kit, and we recommend simply following the kit instructions.

Image: Mixed neuron/glia cultures stained with MAP-2 (green) and also rabbit antibody of [neurofilament NF-H](#) (red). Since the NF-H protein is largely expressed in neuronal axons, while the MAP2 is only found in neuronal dendrites and perikarya, there is little overlap between these two staining patterns. DNA stain shows nuclei of neurons and non-neuronal cells (blue).

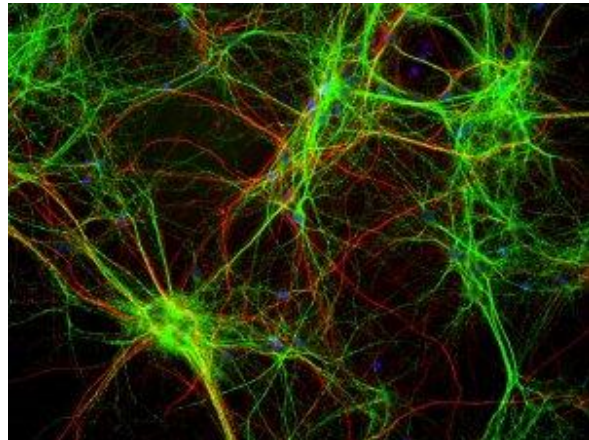


Image: Western blot of whole rat brain lysate probed with antibody to MAP2. Note that the strong single band running at about 280Kda corresponds to MAP2



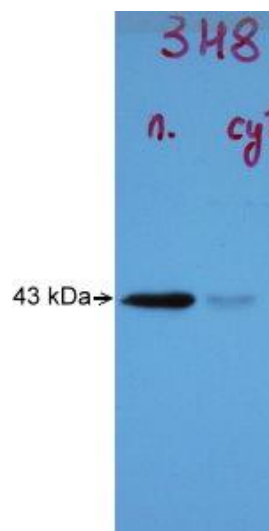
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Images; Blots of TDP43 on crude extract of mouse brain nuclear fraction (left lane) and cytoplasmic fraction (right lane). There is a strong clear band in the nuclear preparation running at 43kDa, and a much more minor band in the cytoplasmic fraction. Image courtesy of Irina Madorsky.



Related Antibodies

Name	Catalog #	Type	Species	Applications	Size	Price
4-Hydroxynonenal (HNE)	GT19004	Goat IgG	H	WB	100 ul	\$295
8-Hydroxydeoxyguanosine (8OHdG)	GT19001	Goat IgG	B; Ca; H; M; P; Pr; R	IHC	100 ul	\$295
Abeta 40 (beta amyloid 1-40)	RA25009	Rabbit IgG	H; M	WB; IP	100 ul	\$255
Abeta 42 (beta amyloid 1-42)	RA25010	Rabbit IgG	H; M	WB; IP	100 ul	\$255
ApoE	MO25042	Mouse IgG	H	IHC; WB; E	100 ul	\$325
APP	RA18003	Rabbit IgG	H; M; Pr; R	IHC; WB	100 ul	\$330
APP 228	MO20015	Mouse IgG	H	IHC	100 ul	\$175
BACE-1	MO15066	Mouse IgG	H; M	IHC; WB; IP; E	500 ug 100 ug	\$325 \$89
BACE-1	GT15136	Goat IgG	H	WB; E	100 ug	\$365
BACE-2	RA25043	Rabbit IgG	H; M; R	IHC; WB	50 ug	\$365
Beta Amyloid	MO20004	Mouse IgG	H	IHC	100 ug	\$150
CREB	MO18006	Mouse IgG	H; M; Pr; R	IF; WB; FC	100 ul	\$275
Presenilin 1	RA18020	Rabbit IgG	H; M; Pr; R	WB; IP	200 ul	\$330
Presenilin 1	GT15027	Goat IgG	H	IHC; WB; E	100 ug	\$365
Presenilin 2	RA18004	Rabbit IgG	H; M; Pr; R	ICC; IHC; WB; IP	100 ul	\$310
alpha Synuclein	MO22111	Mouse IgG	H; M; R	IF; WB	100 ul	\$245

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alpha Synuclein	MO22112	Mouse IgG	H; M; R	IF; WB	100 ul	\$245
alpha Synuclein	MO15053	Mouse IgG	H	WB; E	500 ug	\$325
alpha-Synuclein	GT15112	Goat IgG	H	IHC; WB; E	100 ug	\$365
alpha-Synuclein	RA18021	Rabbit IgG	H; M; Pr; R	WB; IP	100 ul	\$330
TAF15	MO22114	Mouse IgG		H	100 ul	#275
Tau /MAPT+	CH22113	Rabbit IgG	H; R	IF; WB	100 ul	\$245
Tau (Tau 46)	MO18002	Mouse IgG	H; M; R	IF; IHC; WB; IP	100 ul	\$275
Tau	CH23018	Chicken IgY	H; M; R	ICC; IHC	100 ul	\$89
TLR4/CD284	GT15235	Goat IgG	H	IHC; WB; FC; NB	100 ug	\$365
Tyrosine Hydroxylase	MO22941	Mouse IgM	M; Pr; R	IHC; WB	100 ul	\$350
Name	Catalog #	Type	Species	Applications	Size	Price
Tyrosine Hydroxylase	CH23006	Chicken IgY	H; M	ICC; IHC; WB	100 ul	\$99
Tyrosine Hydroxylase	MO20001	Mouse IgG	H; M; R	ICC; WB	100 ul	\$200
Tyrosine Hydroxylase	SO25000	Sheep IgG	H; M; Pr; R	ICC; IF; IHC; WB	100 ul	\$325
phospho-Tyrosine Hydroxylase (Ser40)	RA18026	Rabbit IgG	R	IF; IHC; WB; IP	100 ul	\$335
UCHL1	MO22109	Mouse IgG	B; H; R	IF; WB	100 ul	\$275
UCHL1	MO25040	Mouse IgG	B; H; R	IF; WB	500 ul	\$285
Ubiquitin	MO19005	Mouse IgG	H	IHC; WB	50 ul	\$155
Ubiquitin	MO18001	Mouse IgG	H; M; Pr; R	IHC; WB	100 ul	\$310
Ubiquitin+1	RA15043	Rabbit IgG	H	IHC; WB	100 ug	\$365

Related Recombinant Proteins

Name	Catalog #	Type	Species	Applications	Size	Price
APP/Protease Nexin II (Human), CF	PR15037CF-10	Protein	H; M		10 ug	\$325
ApoE R2, CF	PR15062CF-50	Protein	H		50 ug	\$315
BACE-1 (Human), CF	PR15038CF-50	Protein	H		50 ug	\$325
BACE-1 (Mouse), CF	PR15039CF-50	Protein	M		50 ug	\$325
BACE-2 (Mouse), CF	PR15040CF-20	Protein	M		20 ug	\$325

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Clusterin/APO-J	PR27111-2	Protein	H	2 ug	\$75
				10 ug	\$185
DOPA decarboxylase	PR15053-10	Protein	H	10 ug	\$295
alpha-Synuclein	PR27158-20		H	20 ug	\$85
Ubiquitin	PR27083-20	Protein	H	2 ug	\$85
				100 ug	\$185
WWOX	PR27151-2	Protein	H	2 ug	\$85
				10 ug	\$185

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