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Product datasheet for CH23032-100

MAP2 Chicken Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	IF, IHC
Recommend Dilution:	**Immunocytochemistry:** 1:1000 -1:2000. **Immunohistochemistry**: 1:1000-1:2000.
Reactivity:	Human, Mouse
Host:	Chicken
Clonality:	Polyclonal
Immunogen:	Synthetic peptide (keyhole limpet hemocyanin -KLH conjugate) corresponding two different regions of the MAP2 gene, shared between human (NP_002365, NCBI), mouse (P20357, NCBI) sequences
Formulation:	State: Aff - Purified State: 100 ul Liquid PBS (pH 7.2; 10 mM; isotonic 0.9%, w/v) with sodium azide (0.02%, w/v).
Purification:	Affinity Chromatography
Gene Name:	microtubule associated protein 2
Database Link:	Entrez Gene 4133 Human
Background:	MAP2 belongs to the microtubule-associated protein (MAP) family that also includes MAP1A, MAP1B, MAP1C, MAP4 and tau. These proteins of this family stabilize and promote the assembly of microtubules; which is an essential step in neurogenesis. MAP2 is made up of two ~280kDa apparent molecular weight bands referred to as MAP2a and MAP2b. A third lower molecular weight form, usually called MAP2c, corresponds to a pair of protein bands running at ~70kDa on SDS-PAGE gels. All these MAP2 forms are derived from a single gene by alternate transcription, and all share a C-terminal sequence which includes either three or four microtubule binding peptide sequences, which are very similar to those found in the related microtubule binding protein tau. MAP2 isoforms are expressed only in neuronal cells and specifically in the perikarya and dendrites of these cells. Antibodies to MAP2 are therefore excellent markers on neuronal cells, their perikarya and neuronal dendrites. In contrast tau is found predominantly in neuronal axons
Synonyms:	Microtubule-associated protein 2, MAP2, Neuronal Marker
Note:	Protocol: **Immunostaining Cell Cultures** 1. Draw of culture medium with aspirator and add 1 ml of 3.7 % formalin in PBS solution to

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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 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 mls of secondary antibody. These are fluorescently labeled Goat anti mouse or rabbit 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). For anti-fading, use Neuromics' i-BRITE Plus –Catalog#: SF40000 or make your own 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 phenylene diamine is completely dissolved, add an equal volume of glycerol and mix. This reagent will last about a week at -20OC. Discard this reagent when it starts to turn dark brown.

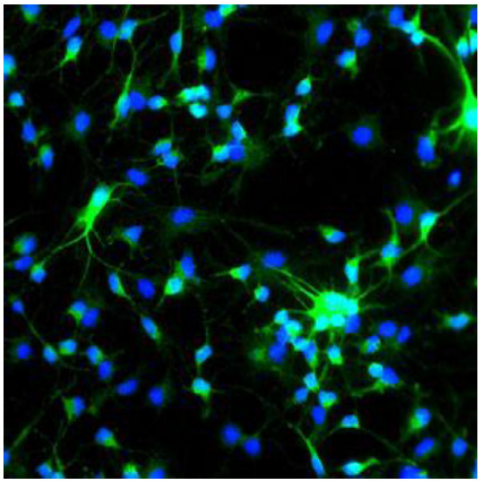
Other Reagents

Fluorescein-labeled goat anti-chicken IgY

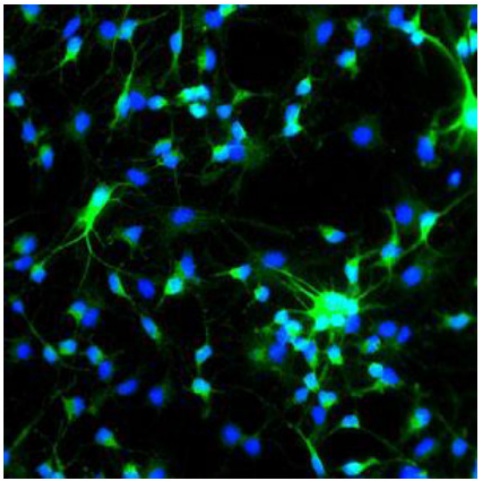
1. Prepare your tissue sections or cultured cells as you normally would. Wash your sections or cells for 1 min with PBS at room temperature.
2. Incubate your sections or cells with your chicken primary antibodies (diluted in "antibody dilution buffer") for at least 1 hour at room temperature. The concentration 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-chicken IgY (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).

Product images:



MAP2 positive cultured neurons (green) from an e13.5 mouse brain. Neuclei are in blue.



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