NEUROMICS

GFAP Data Sheet

Catalog Number:	RA22101	**Host:**	Rabbit
Product Type:	Polyclonal antiserum	**Species Reactivity:**	Rat, Mouse
Immunogen Sequence:	Recombinant GFAP and purified bovine GFAP	**Format:**	Whole serum

Applications: **Immunohistochemistry:** 1:1000; 1:5000 using ABC amplification

Western Blot: 1:50,000

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.*

References: Paula G. Franco, Juana M. Pasquini, Lucas Silvestroff. Optimizing Culture Medium Composition to Improve Oligodendrocyte Progenitor Cell Yields In Vitro from Subventricular Zone-Derived Neural Progenitor Cell Neurospheres. Published: April 2, 2015DOI: 10.1371/journal.pone.0121774

David H. McDougal,Gerlinda E. Hermann, and Richard C. Rogers. Vagal Afferent Stimulation Activates Astrocytes in the Nucleus of the Solitary Tract Via AMPA Receptors: Evidence of an Atypical Neural–Glial Interaction in the Brainstem. The Journal of Neuroscience, 28 September 2011, 31(39): 14037-14045; doi: 10.1523/JNEUROSCI.2855-11.2011

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 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!

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Immunostaining Tissue

Solutions

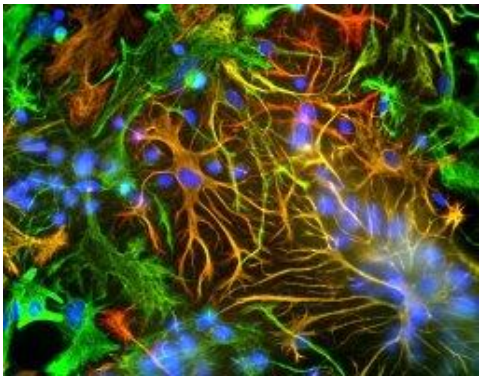
PBS - sodium phosphate-buffered (100 mM; pH 7.2) isotonic (0.9% NaCl, w/v) saline Antibody dilution buffer (PBS with 0.1% non-ionic detergent, such as Triton X-100 or Tween-20) fluorescein anti-fading reagent -- Make up a 2 mg/ml phenylene diamine solution in PBS (phenylene diamine requires extensive vortexing to put it into solution). Once the phenylene diamine is completely dissolved, add an equal volume of glycerol and mix. This reagent will last about a week at -20^{O}C. Discard this reagent when it starts to turn dark brown.

Other Reagents

Fluorescein-labeled goat anti-rabbit 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-rabbit 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).

Image: Mixed neuron-glial cultures stained with rabbit GFAP (red channel) and chicken vimentin (green channel). The fibroblastic cells contain only vimentin and so are green, while astrocytes contain either vimentin and GFAP, so appearing golden, or predominantly GFAP, in which case they appear red. Blue is nuclear DNA stain



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