Enriched Neuronal Morphology Revealed By Monoclonal Cocktail Immunostaining

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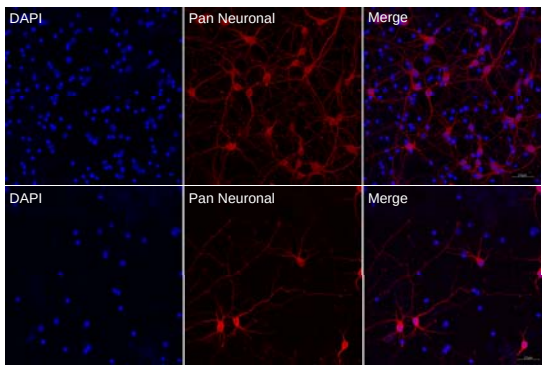
Introduction

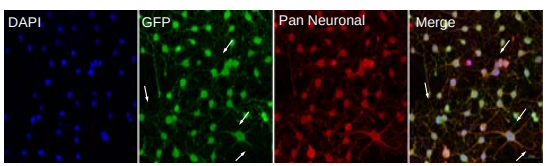
Antibodies to neuronal proteins have become critical tools for identifying neurons and discerning morphological characteristics in culture and complex tissue. While the labeling from classic histological techniques such as Golgi staining and modern molecular approaches such as GFP constructs yield excellent cytoarchitectural detail, these approaches are technically challenging and impractical for many neuroscience research needs. Neuron-specific antibodies are convenient precision tools useful in revealing cytoarchitecture, but are limited to the protein target distribution within the neuron, which may differ greatly from nucleus to soma to dendrite and axon.

To achieve as complete a morphological staining as possible across all parts of neurons, we have developed a monoclonal antibody blend that reacts against key somatic, nuclear, dendritic, and axonal proteins distributed across the pan-neuronal architecture that can then be detected by a single secondary antibody. The Milli-Mark™ pan-neuronal antibody (Catalogue number MAB2300) is the first release in a line of monochromatic and polychromatic enhanced neuronal markers validated in diverse protocols and tissues, giving researchers a convenient and specific qualitative and quantitative tool for studying neuronal morphology.

MAB2300 staining in mixed primary cortical culture.



Milli-Mark Pan Neuronal Marker: Neuro differentiated GFP-construct labeled rat hippocampal stem cells. Note how MAB2300 reveals previously weakly labeled features.



Methods

Millipore's Milli-Mark™ Pan Neuronal antibody blend (Catalogue number MAB2300) creates a tool whereby one reagent can be used by a researcher to label (with one secondary) any neuronal type as completely as possible, eliminating the need for multiple antibodies or concern for weak staining that occurs using some single immunogen 'pan'-neuronal antibodies for certain neuron types. This antibody cocktail has been validated in diverse fixations and immunohistochemistry protocols, including archival tissue.

Simple Milli-Mark™ Pan Neuronal Immuno Protocols

Immunocytochemistry (ICC):

1. Culture neurons as desired. When ready, gently wash with PBS and fix cells with 4% paraformaldehyde in PBS, 30min at room temp (RT)

2. Block with blocking buffer 2.5% BSA, 5% donkey serum , 0.1% Triton x-100 in PBS.

3. Wash with PBS, 3 x 5min each, at RT and block with blocking buffer (same as above) for 1hr at RT

4. Incubate cells with Milli-Mark™ pan Neuronal (Mouse IgG) (Cat# MAB2300) for 2hr at RT (dilute antibody in blocking buffer)

6. Wash with PBS, 3 x 5min each, and incubate cells with secondary antibody for 1hr at RT (Antibodies validated with Milli-Mark™ Pan Neuronal to produce minimal background include: Dky x Ms-Cy3 (Cat# AP192C); Dky x Ms-FITC (Cat# AP192F); Gt x Ms-Cy3 (Cat# AP181C); Gt x Ms-FITC (Cat# AP181F);1:1000 in blocking buffer)

7. Wash with PBS, 3 x 5 min each, and cover slip with Aqua poly/mount. View using fluorescent microscope.

Immunohistochemistry (paraffin):

1. Deparaffinize and hydrate tissue sections through xylenes and other clearing agents and graded alcohol series

2. Rinse for 5 min in PBS at room temp (RT). Then incubate section in 0.3% H_2O_2 for 30min at RT to quench endogenous peroxidase activity

3. Wash in PBS 5 min and block in blocking buffer, 1hr at RT

4. Incubate sections in Milli-Mark™ Pan Neuronal Ab, 1:25 in blocking buffer, overnight at 4˚ C

5. Wash in PBS 3x5min and incubate sections for 30min at RT with diluted biotinylated secondary antibody solution

6. Wash in PBS 3x5min and incubate sections for 30min at RT with avidin/biotinylated enzyme complex

7. Wash in PBS 3x5min and incubate sections in DAB/Nickel solution (for 2-10min) until suitable staining develops

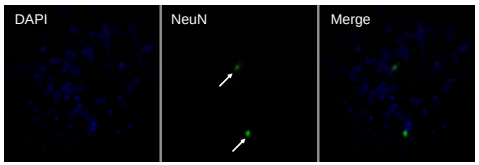
8. Wash with PBS, dehydrate and cover slip with non-aqueous mounting media

Results – Development of Pan Neuronal Marker Cat. # MAB2300

Individual antibodies, while specific, are not broadly staining.

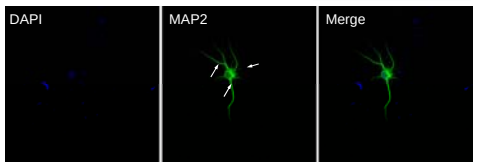
NeuN staining in mixed primary cortical culture. 10ug/mL

Cellular localization	
Dendrites	-
Cell body	-
Nuclear	++++
Axon	-



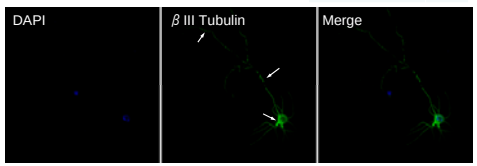
MAP2 staining in mixed primary cortical culture. 10µg/mL

Cellular localization	
Dendrites	++++
Cell body	+++
Nuclear	-
Axon	-/+



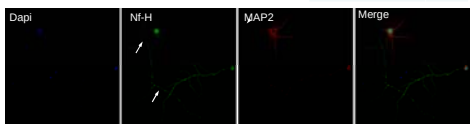
β III Tubulin staining in mixed primary cortical culture. 10µg/mL

Cellular localization	
Dendrites	+
Cell body	+++
Nuclear	-
Axon	++++



NF-H staining in mixed primary cortical culture. 10µg/mL

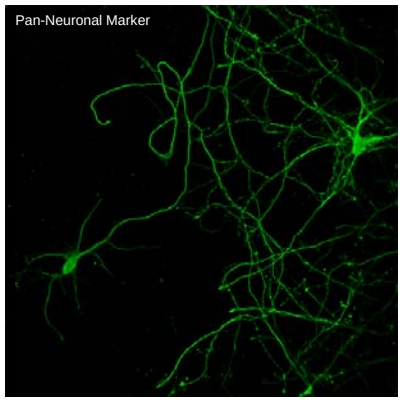
Cellular localization	
Dendrites	-
Cell body	-
Nuclear	++
Axon	++++

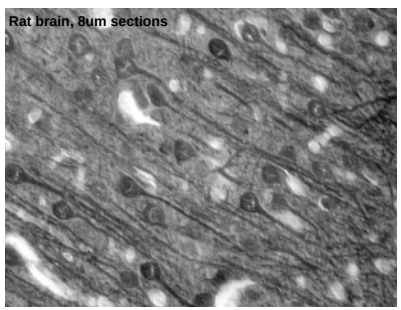


Millipore's Milli-Mark™ Pan Neuronal antibody is a blend of exclusive proprietary antibodies optimized for complete neuronal staining.

MAB2300 staining in mixed primary cortical culture. 10ug/ml.

Cellular localization	
Dendrites	++++
Cell body	++++
Nuclear	++++
Axon	++++





Summary

- The Milli-Mark™ pan-neuronal antibody (Catalogue number MAB2300) gives researchers a convenient and specific qualitative and quantitative tool for studying neuronal morphology.
- The Milli-Mark™ line of research antibodies are highly-validated in multiple protocols, tissues and species.
- MAB2300 has been shown to reveal significantly better neuronal detail than traditional neuronal antibody markers and better morphological resolution than GFP-construct labeled neurons.
- MAB2300 has been tested successfully in numerous neural cell lines, including: PC12, SHSY5Y, N1E-115 Neuroblastoma

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