Molecular Targeting Technologies, Inc.

M · T · T · I

NeuroVue® Dye Filters for Neuronal Tract Tracing

What are they?

NeuroVue®Dye Filters are useful tools in several different areas of research including neuronal tract tracing studies of up to 3-4 weeks and are spectrally compatible with most fluorescent light-absorbing tags.

What do NeuroVue Dye Filters Offer?

- Convenient, ready-to-use coated filter format
- More precise control of dye insertion point
- No messy oils, pastes or hard-to-position crystals
- Diffusion properties comparable to or better than other commercially available neurotracing dyes
- More focal results (e.g. labeling of small sets of axons within pathway)
- Available in multiple colors, including far red, for multi-tract tracing and improved results even in tissues with high myelin expression

How do they work?

NeuroVue®Dye Filters have been found to be useful for tracing neuronal connections in animal tissues fixed in formaldehyde. Like other lipophilic tracers, they readily transfer into plasma membranes in fixed tissues and diffuse laterally within the membrane, eventually labeling the entire cell body as well as the finest axonal and dendritic branches and allowing visualization of neuronal processes up to several millimeters distant from the point of dye insertion.

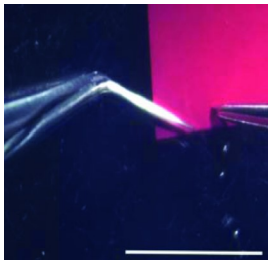

Figure 1: Preparation of NeuroVue®Red micro-strips for use in tissue labeling. Using a dissecting microscope, micro-scissors are used to cut small triangles from a 1 x 1 cm coated filter square. Magnification ~25X

NeuroVue®Dye Filters are provided in coated filter format because insertion of small dye coated filter segments have been shown to be a simple, reliable method for labeling well defined tissue regions, avoiding known artifacts associated with labeling via high pressure microinjection or insertion of dye crystals on a dissecting needle.
NeuroVue®Dye Filters can be selected such that they exhibit minimal bleed through into filter windows typically used for other fluorescent probes making them an excellent choice for multicolor neurotracing studies in sections and/or whole-mount preparations.

Overview of Labeling Strategy

- Fix tissue in 4% buffered formaldehyde.
- Initiate labeling by inserting NeuroVue®micro-strip(s) (Figure 1) into nerve tract(s) to be traced (Figure 2). The highly lipophilic NeuroVue®dyes transfer from the micro-strip into nerve cell membranes and diffuse along the lipid bilayer in both directions from the insertion site (anterograde and retrograde labeling).
- Incubate tissue in 4% phosphate buffered formaldehyde at 37°C.
- Monitor the progress of dye diffusion using light microscopy and/or fluorescence microscopy. (Figure 3)
- When dye(s) have reached the region(s) to be studied, remove NeuroVue®micro-strip(s) and prepare whole mounts or tissue sections for fluorescence imaging. (e.g. Figure 4)

Advantages of NeuroVue®Technology

- Different fibers can be traced in the same specimen by using fluorescent NeuroVue®dyes that excite and emit in the green, orange, red and/or far red.

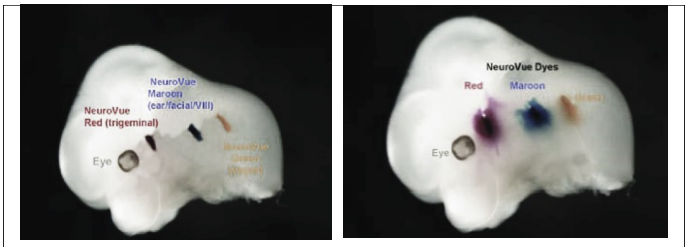

Figure 2: Placement of NeuroVue®micro-strips for multicolor neurotracing. Lateral view of murine head (embryonic day 12.5); with micro-strips placed to obtain central projections of NeuroVue®Red labeled trigeminal nerve, NeuroVue®Maroon labeled facial nerve and NeuroVue®green labeled glossopharyngeal nerve. The eye is visible as a brown spot at left (anterior). Magnification ~25X. NeuroVue®Jade is currently recommended for such studies because it can be visualized over substantially longer distances than NeuroVue®Green.

Figure 3: Monitoring diffusion distance using NeuroVue®dye absorbance. After incubation for 36 h at 37°C, diffusion in all directions from the point of micro-strip insertion is readily visualized using a dissecting microscope (same specimen as Figure 2) Magnification ~25X

Photos courtesy of Drs. Bernd Fritzsch and Lucy Feng (Creighton University)

- Neuronal connections can be studied in embryos lacking receptors needed for neuronal identification as well as in juveniles and adults. (Gurung & Fritzsch, J Comp Neurol 479:309-327, 2004; Morris et al., Brain Res 1091:186-199, 2006; Hsieh & Cramer, J Comp Neurol 497:589-599, 2006)
- Use of dye-coated filters allows more precise positioning than is possible with crystals or oils, avoids tissue damage caused by high pressure microinjection and provides sharp high resolution images of both afferent and efferent fibers arising at the point of filter insertion. (Fritzsch et al., Brain Res Bull 66:249-258, 2005; Kikkawa et al., Acta Otolaryngol Suppl., Dec (559) 19-23, 2007)
- Use of NeuroVue®dyes reduces the complexity of labeling procedures because these dyes have been selected to have similar diffusion rates, allowing simultaneous or near-simultaneous application of different colors in most cases. (Jensen-Smith et al., Immunol Inv. 36, No 5-6, 763-789, 2007)

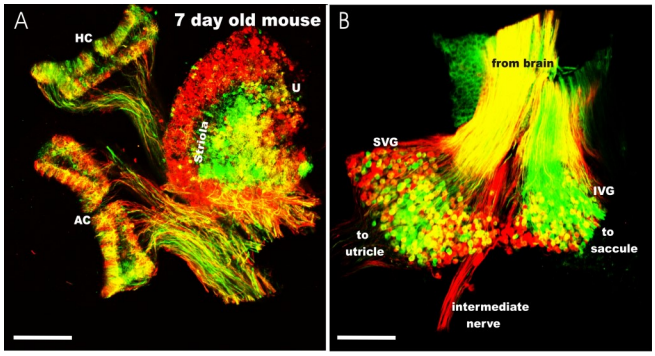

Figure 4: Simultaneous labeling of afferent and efferent fibers in the vestibular system using NeuroVue® Maroon and NeuroVue®Red. After perfusion fixation, NeuroVue® Maroon (green) was injected into the cerebellum and NeuroVue®Red into the superior vestibular nucleus of a 7 day old juvenile mouse and dyes were allowed to diffuse for 4 weeks at 36°C. Labeled fibers to the three vestibular end-organs (utricle, U; anterior crista, AC; horizontal crista, HC) were readily visualized (Panel A) and both overlap and segregation were seen for both sets of fiber types in a given end-organ (*e.g.*, U in Panel A). Neurons of the vestibular ganglion exhibited some clustering but showed largely an intermingled distribution (Panel B). [Beisel et al. Journal of Vestibular Research, 15:225-241, 2005.]

For further information or to place an order please contact Dr. Brian Gray by email (briangray@mtarget.com), phone: 610-738-7938 or fax: 610-738-7928.