

# Euro-Glo®

Euro-Glo represents the first fluorescent metal chelate to be used as a histochemical tracer, specifically to localize normal and pathological myelin as well as amyloid plaques.

## HIGHLIGHTS

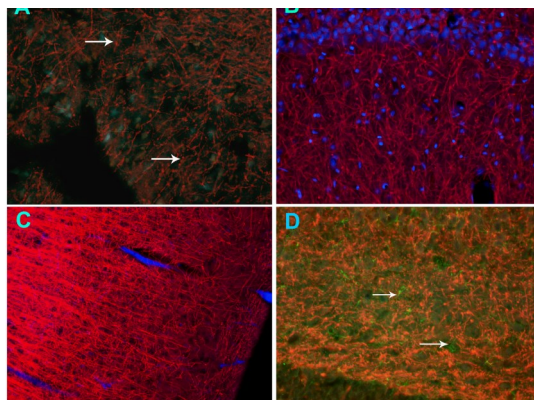
Euro-Glo is a novel fluorochrome consisting of an organic chelate of the rare earth metal Europium.

Euro-Glo allows for fluorescent localization of myelinated fiber bundles, individual fibers and toxicant induced myelinopathies.

Euro-Glo staining also labels amyloid plaques, with the surround appearing red and the core appearing blue in color.

Comparing Euro-Glo staining with a number of well characterized histochemical tracers suggests that it may have an affinity for glycolipids.

Euro-Glo is the first rare earth metal chelate to be applied histologically for the study of the brain.



## ABSTRACT

**Background:** The vast majority of fluorochromes are organic in nature and none of the few existing chelates have been applied as histological tracers for localizing brain anatomy and pathology.

**New Method:** In this study we have developed and characterized a Europium chelate with the ability to fluorescently label normal and pathological myelin in control and toxicant-exposed rats, as well as the amyloid plaques in aged AD/Tg mice.

**Results:** This study demonstrates how Euro-Glo can be used for the detailed labeling of both normal myelination in the control rat as well as myelin pathology in the kainic acid exposed rat. In addition, this study demonstrates how E-G will label the shell of amyloid plaques in an AD/Tg mouse model of Alzheimer's disease a red color, while the plaque core appears blue in color. The observed E-G staining pattern is compared with that of well characterized tracers specific for the localization of myelin (Black-Gold II), degenerating neurons (Fluoro-Jade C), A-beta aggregates (Amylo-Glo) and glycolipids (PAS).

**Comparisons with existing methods:** This study represents the first time a rare earth metal (REM) chelate has been used as a histochemical tracer in the brain. This novel

tracer, Euro-Glo (E-G), exhibits numerous advantages over conventional organic fluorophores including high intensity emission, high resistance to fading, compatibility with multiple labeling protocols, high Stoke's shift value and an absence of bleed-through of the signal through other filters.

**Conclusions:** Euro-Glo represents the first fluorescent metal chelate to be used as a histochemical tracer, specifically to localize normal and pathological myelin as well as amyloid plaques.

## METHODS

Following euthanasia with Euthasol, animals were perfused via the ascending aorta with 10% formalin in 0.1 M neutral phosphate buffer. The brains were removed and post-fixed overnight in the same fixative solution plus 20% sucrose for cryoprotection. The brains were then cut at a thickness of 25  $\mu$ m on a freezing-sliding microtome. The brain tissue sections were either collected in wells containing 0.1 M neutral phosphate buffer for short term storage in the refrigerator, or in wells containing antifreeze (ethylene glycol: glycerin: distilled water: 0.1 M neutral phosphate buffer 1:1:1:1) for long term storage in the freezer. After two 3 min rinses in distilled water, the tissue sections were mounted onto gelatin coated slides from distilled water and allowed to dry on a slide warmer at 55° C for at least 30 min.

Slide mounted tissue sections were first re-hydrated by immersion in distilled water for 5 min. The slides were then transferred to a 0.025% solution of Euro-Glo dissolved in a distilled water vehicle for 1-4 days at room temperature. The progression of the stain may be microscopically monitored daily until optimal staining is achieved. Once fully stained, the slides were then given a 3 min

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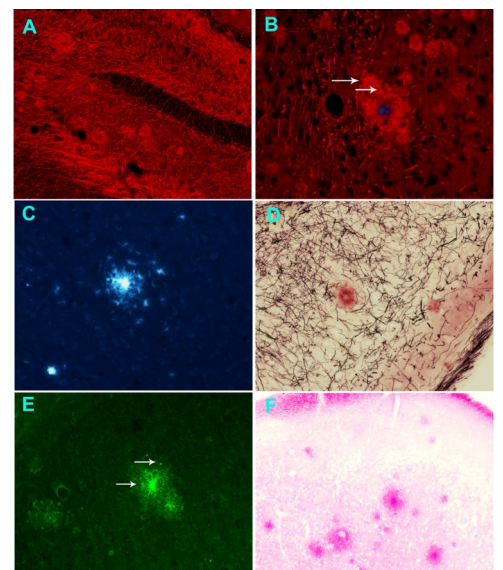
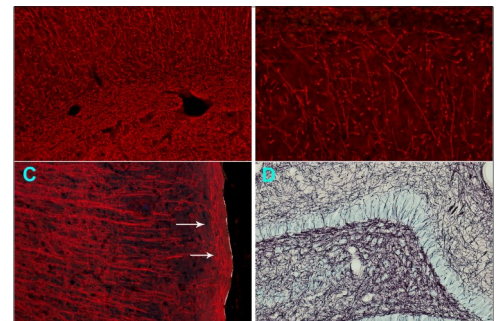


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rinse in distilled water and then transferred to an ammonium borate solution for 1 min. Following a distilled water rinse, the progression of the differentiation can be monitored microscopically and the slide returned to the ammonium borate solution if the background remains too high. However, differentiation times in excess of 6 min are rarely needed and longer times can potentially bleach the specific labeling. To avoid fading under UV examination, the tissue should be well rinsed as indicated below. The ammonium borate differentiating solution was made by dissolving 4 g of boric acid and 10 ml of concentrated (40%) ammonium hydroxide in 90 ml of distilled water. Should the slides become over differentiated, they may be re-stained in the Euro-Glo solution. Once differentiated, the slides were rinsed through three 5 min distilled water rinses. Insufficient rinsing of differentiating solution can result in fading under examination. Slides were then coverslipped with an aqueous non-fluorescent mounting media, Ever-Glo, (Histo-Chem, AR) consisting of polyvinyl alcohol, glycerin and borate buffer. Polar mounting media such as DPX and Permount are not suitable for coverslipping E-G stained tissue. Other aqueous mounting media such as Prolong-Gold (Thermo Fisher, CA) were found suitable for short term examination but allowed the section to fade from the edges inward within a few days.



## REFERENCE

Supplemental staining procedures can be found in the following reference:

Larry Schmued, James Raymick, *Journal of Neuroscience Methods* 279, Introducing Euro-Glo, a rare earth metal chelate with numerous applications for the fluorescent localization of myelin and amyloid plaques in brain tissue sections (2017) 79-86.

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