

# FLUOROCHROME, LLC

5285 East Sixth Avenue  
Denver, Colorado 80220 USA  
Telephone: (303) 394-1000

e-mail: [info@Fluorochrome.com](mailto:info@Fluorochrome.com)  
website: [www.Fluorochrome.com](http://www.Fluorochrome.com)

## Fluoro-Ruby Protocol and Product Information

Fluoro-Ruby is a quick, sensitive, reliable, and technically simple fluorescent anterograde tracer and marker. The use of Fluoro-Ruby is essentially the same as other fluorescence tracers. Following is a suggested protocol:

### Fluoro-Ruby Protocol:

A 10% solution of Fluoro-Ruby is made by dissolving 10 mg of Fluoro-Ruby dry powder in 100  $\mu$ L of distilled water. It is then delivered via intracranial stereotaxic injection using a one  $\mu$ L Hamilton microsyringe to pressure inject the tracer. Injection volumes typically range from 0.02-0.1  $\mu$ L and are gradually injected over a 10-15-minute interval. The animal is allowed to recover and is sacrificed typically 4-14 days later.

The animal is then perfused with neutral buffered formaldehyde (10% formalin or 4% paraformaldehyde in 0.1 M neutral phosphate buffer.) The brain is removed and postfixed for at least overnight in the same fixative solution plus 20% sucrose for cryo-protection. The brain is then cut on a freezing sliding microtome or cryostat into sections usually between 20 and 40 microns in thickness.

The tissue sections are mounted on gelatin coated slides and are air dried on a slide warmer at 50-60 degrees Centigrade for at least one-half hour. The dry slides are then transferred to xylene clearing solution for at least one minute and then coverslipped with DPX mounting media. The slides can then be examined under an epifluorescent microscope using a filter suitable for visualizing TRITC (green light excitation).

### Fluoro-Ruby Product Information:

**Compound:** Fluoro-Ruby

**Classification:** Tetramethylrhodamine dextran-amine

**Appearance:** Pink to crimson colored powder

**Solubility:** Highly soluble in aqueous solutions

**Purity:** Thin layer chromatography using cellulose plates and a solvent system of acetonitrile and water (6:4) revealed no detectable free TRITC dye.

**Substitution:** One molecule TRITC per molecule of dextran-amine

**Average Molecular Weight:** 10,500

**Excitation Peak:** 540 nm

**Emission peak:** 600 nm

**Filter system for visualizing:** Standard TRITC filter

**Storage:** The dry powder can be stored in a tight container and may be stored at room temperature or in a commercial refrigerator without the frost-free feature. Solutions should be tightly covered and refrigerated when not in use.

**Toxicity:** Although the compound appears to be of low toxicity, it has not been extensively evaluated and therefore routine laboratory caution should be exercised. Fluoro-Ruby is not approved for human consumption.

### References:

Schmued, L., Kyriakidis, K., & Heimer, L., In vivo anterograde and retrograde axonal transport of the fluorescent rhodamine-dextran-amine, Fluoro-Ruby, within the CNS. *Brain Res*, 526 (1990) 127-134.

Bowyer, J.F. and Schmued, L., Fluoro-Ruby labeling prior to an amphetamine neurotoxic insult shows a definitive massive loss of dopaminergic terminals and axons in caudateputamen. *Brain Res.*, 1075, (2006) 236-239.