# Molecular Probes Fluorescent Nissl Stains

These labels bind RNA to label rough endoplasmic reticulum (Nissl bodies in neurons). Nucleoli and other nuclear RNA will be visible as well. Degree of DNA binding is unknown but these produce excellent nucleolar labelling. Molecular Probes has several of these dyes with overlapping spectra for excitation and emission. The two stains described here appear to have the most constrained spectra for minimal bleedthrough when used with multiple fluorescent labels.

These should be applied after immunocytochemical labeling. The Triton X-100 rinses before and after labeling appear to increase contrast. The Triton X-100 pre-wash is not needed after immuno-labeling that uses detergent in any step. The 20 minute incubation recommended by Molecular Probes is sufficient for paraffin or cryostat sections. A 60 minute incubation ensures penetration through the full thickness of 40 $\mu$m vibratome sections but does not increase the staining intensity. The Deep Red appears to penetrate sections more slowly than the Green.

|  | Catalog | Em. | Ex. |
|---|---|---|---|
| NeuroTrace Green | D21480 | 500 nm | 525 nm |
| NeuroTrace Deep Red | D21483 | 640 nm | 660 nm |

Procedure for Sections:
1. Incubate sections 10 min. in 0.1% Triton X-100 in PBS (optional);
2. Dilute NeuroTrace Green (N21480) or Deep Red (N21483) with PBS, 1/150 for confocal or 1/300 for epifluorescence;
3. Incubate sections 40 min. (Green) or 60 min. (Deep Red) for 40 $\mu$m vibratome, 20 min. for other section types of 12 $\mu$m or less;
4. Wash briefly with PBS;
5. Incubate sections for 10 min. with 0.1% Triton X-100 in PBS;
6. Rinse sections 3 times in PBS for 10 min. each.
7. Coverslip with 1% DABCO in 90% glycerol.

Epifluorescence Microscopy.
View NeuroTrace Green with standard filters for FITC or GFP. The Deep Red requires a Cy5 filter cube and a digital camera capable of detecting in the near IR.

Confocal Microscopy.
Excite NeuroTrace Green with a 488 nm laser line and collect emissions through any filter suitable for green fluorescence. The NeuroTrace Deep Red excites equally well with the 568 nm and the 647 nm lines. Set a channel to collect the Deep Red signal from 640 nm or 650 nm and beyond.