Protocol 1, Jour of Comp Neurology 503:454-465 (2007)

mice E13.5-16.5

・Fix with 4% PFA/0.1 M PB pH 7.4 ON

・Embed in 2% agarose and cut by using a vibrating microtome (Microslicer) from caudal direction to expose the cerebral and thalamic primordial

・Apply DiI crystals on the cortical surface and dorsal thalamus

・Incubate the tissue blocks in 4% PFA in the dark at 37°C for 1-2 weeks

・Cut coronal 100-μm-thick sections on a Microslicer and observe on a laser-scanning microscope (Olympus, FV1000)

Protocol 2, Hippocampus 16:437-442 (2006)

rat P8 (slice culture)

・Prepare 350-μm-thick sections using a sliding vibratome in culturing buffer at 4°C

・Culturing

・Fix sections with 4% PFA/0.1 M PB for 5 days on the cell culture insert at RT and then wash in 0.1 M PB (3×10 min)

・Remove fixative

・Apply a minute crystal of DiI to the slice under microscopic control and store in RT for 3-10 days

・Wash the slice with PB (10 min) and coverslip with DAKO fluorescent mounting medium

・Analyze by confocal laser scanning microscope

Protocol 3, Auton Neurosci 105:131-144 (2003)

human brainstem specimen (postmortem)

・Fix the whole tissue by immersion in 4% PFA/0.1 M PB containing 4% sucrose and 0.05% sodium azide, pH 7.4) at 4°C for 24 h

・Cut the specimen by appropriate blocks

・Dissectiong microscope, apply a crystal DiI approximately 0.5 mm in diameter, with a hypodermic needle to the block

・Store the block in the same fixative at RT protected from light for 6 to 16 months

・Place the block in 30% sucrose in 0.1 M PB for 24 h

・Freeze the block on dry ice and section at 40-μm-thick

・Wash the section with 0.1 M PB

・Mount the section on uncoated glass slide and cover slippe in the buffer

・Observe the section with a fluorescent microscope