SUPPLEMENTAL PROTOCOL

NEUROBIOTIN® Tracer for Intracellular Labeling of Neurons

This procedure was kindly supplied by Dr. Hitoshi Kita,
Univ. of Tennessee, Dept. Anatomy & Neurobiology, Memphis, TN 38163

A. Intracellular recording electrode:

Glass micropipetes were pulled from capillaries (1–2 mm, O.D.) containing a microfilament. They were filled with 2% NEUROBIOTIN Tracer in 1.0 M potassium methylsulfate or 1.0 M potassium chloride. The resistance of the electrodes measured in Ringer solution ranged from 60–150 MΩ.

B. Intracellular injection:

Neurons in in vitro brain slice preparations or anesthetized animals were impaled with the recording electrodes and were injected with NEUROBIOTIN Tracer by passing 1–5 nA depolarizing rectangular pulses of 150 ms duration at 3.3 Hz for 2–10 min.

C. Staining sections:

Brain slices were fixed by submersion in 4% paraformaldehyde and 0.2% picric acid in 0.15 M phosphate buffer (pH 7.4) overnight. For in vivo experiments, anesthetized animals were fixed by perfusion of saline followed by the same fixative through the left ventricle. Conventional Vibratome or frozen sections (40 µm thick) were cut from the brain tissue and collected in phosphate buffered saline (PBS, pH 7.3). After several rinses with PBS, sections were treated with Triton X-100 (0.4% in PBS) for 1 to 2 hours and then incubated in the VECTASTAIN® ABC Reagent in PBS for 2 h. After several rinses with PBS, they were reacted with diaminobenzidine (DAB 0.05%) and H_2O_2 (0.003%) in PBS to visualize injected neurons*. The sections were mounted onto gelatin-coated slides, dried, defatted, and coverslipped. Some of the DAB-reacted sections were post-fixed with 0.5% osmium tetroxide for intensification of the DAB reaction product before mounting on slides.

*There are alternative methods for visualizing injected neurons. For example, the Triton X-100 treated sections can be incubated:

a) in avidin, then biotin conjugated markers (e.g., horseradish peroxidase, etc.) or

b) in avidin conjugated marker. (e.g., fluorescein, Texas Red™, etc.)

Following this step, the sections can be further processed for other treatments, such as immunocytochemistry for neuroactive substances.

Kita H, et al. 1991. A Biotin-Containing Compound N-(2-Aminoethyl) Biotinamide for Intracellular Labeling and Neuronal Tracing Studies: Comparison with Biocytin. *Journal of Neuroscience Methods.*

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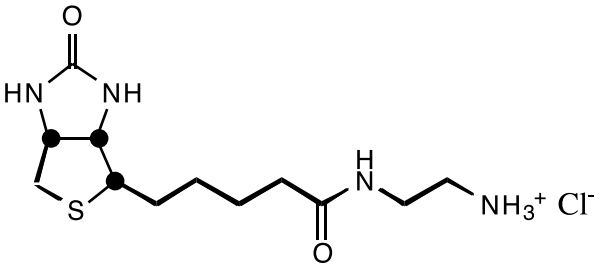
Product Specifications

Product	NEUROBIOTIN® Tracer
Cat. No.	SP-1120
Amount	50 mg
Lot No.	F0921
Storage	2–8°C (desiccated). Once in solution, store frozen.
Empirical formula	$C_{12}H_{23}ClN_4O_2S$
Molecular Weight	322.8
Structure	

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