



NEUROBIOTIN™ Tracer for Intracellular Labeling of Neurons

This procedure was kindly supplied by
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A. Intracellular recording electrode:

Glass micropipettes 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 nA to 5 nA depolarizing rectangular pulses of 150 ms duration at 3.3 Hz for 2 to 10 minutes.

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-X100 (0.4% in PBS) for 1 to 2 hours and then incubated in the VECTASTAIN® ABC Reagent in PBS for 2 hours. After several rinses with PBS, they were reacted with diaminobenzidine (DAB 0.05%) and H₂O₂ (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 TritonX100 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.

H. Kita & W. Armstrong: A biotin-containing compound N-(2-aminoethyl) biotinamide for intracellular labeling and neuronal tracing studies: Comparison with biocytin. *J. Neurosci. Methods.* 37, 141-150, 1991.

For a complete list of references on Neurobiotin™ Tracer please contact Vector Laboratories, Inc.



PRODUCT SPECIFICATIONS

Product NEUROBIOTIN™ Tracer
N-(2-aminoethyl) biotinamide hydrochloride

Catalog No. SP-1120

Amount 50 mg

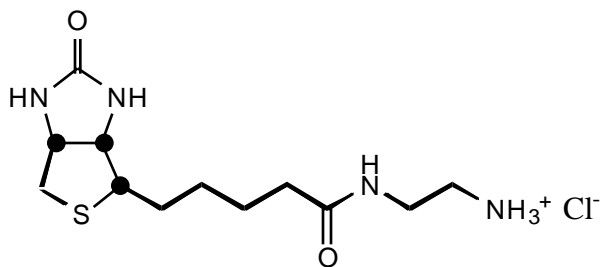
Lot No. F0921

Storage 4°C (desiccated)

Empirical formula $C_{12}H_{23}ClN_4O_2S$

FW 322.847

Structure



See accompanying instructions for use.