USER GUIDE

NEUROBIOTIN® 488 Tracer

Guidelines for Intracellular Labeling

vector LABORATORIES

Together we breakthrough™

Cat. No. **SP-1125**

Storage 2-8 °C (desiccated). Once in solution, store frozen.

Injection

Intracellular labeling with a recording pipet: Patch-clamp recording conditions were kindly provided by Andrei Derbenev, Ph.D., Department of Physiology, Tulane University. Electrodes with a resistance of 2–4 MΩ were filled with 0.1 % or 0.5 % NEUROBIOTIN 488 in 130 mM Cs gluconate, 10 mM HEPES, 1 mM NaCl, 5 mM EGTA, 1 mM $MgCl^2$, 1 mM $CaCl^2$, 2 mM ATP, (adjusted to pH 7.2 using CsOH). Cell membranes were ruptured and recordings of individual neurons in 400 µm mouse brain sections were made for about 10 minutes. NEUROBIOTIN 488 diffused into the cell during recording. The section was then fixed with 4% paraformaldehyde, mounted on a charged slide and viewed by fluorescence microscopy.

Pressure injection

Successful tracing has been accomplished by pressure injection of 100 nl of 5% NEUROBIOTIN 488 in 3 M Tris, pH 8.3.

Detection

Visualization of the signal can be accomplished either by the fluorescence or the biotin of the NEUROBIOTIN 488.

Fluorescence

Fluorescence can be directly visualized by standard fluorescence microscopy.

Biotin

Many options are available for detection of biotin in tissue sections. The following procedure is provided as a guideline utilizing a peroxidase detection system.

1. Following formaldehyde or glutaraldehyde fixation, air dry sections. If endogenous peroxide activity is present, inactivate using BLOXALL® Endogenous Peroxidase/Alkaline Phosphatase Blocking Solution (Cat. No. SP-6000).
2. Perform biotin blocking, if required, using an Avidin/Biotin Blocking Kit (SP-2001) or a Streptavidin/Biotin Blocking Kit (SP-2002). Block non-specific binding by incubating section with Normal Serum for 30 minutes at room temperature. Blot excess blocking solution from the sections.
3. Prepare the VECTASTAIN® Elite® ABC Reagent (Cat. No. PK-6100) according to the kit instructions. Apply to the sections and incubate at room temperature. Incubate for several hours for 40 µm sections. A 24 hour incubation is usually adequate for 400 µm sections. Wash thoroughly with PBS containing 0.1% Tween® 20.
4. Apply a precipitating peroxidase substrate such as ImmPACT® DAB (Cat. No. SK-4105), ImmPACT® VIP (Cat. No. SK-4605), or ImmPACT® SG (Cat. No. SK-4705). Rinse in water.
5. Counterstain (optional), clear, and mount.

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