

EZ-Link[®] Biocytin

28022

0450.3

Number

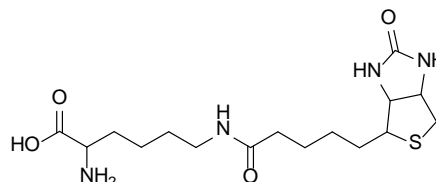
28022

Description**EZ-Link Biocytin**, 100mg

Spacer Arm: 20.1Å

CAS Number: 576-19-2

Molecular Weight: 372.48

**Storage:** Upon receipt store at room temperature. Product shipped at ambient temperature.**Introduction**

The Thermo Scientific EZ-Link Biocytin consists of biotin conjugated to the epsilon amine of lysine and is formally referred to as (ϵ -N-[d-biotinyl]-L-lysine). Biocytin is a naturally occurring molecule present in serum and urine, which has high binding affinity for avidin and streptavidin and can be used as an intermediate in the synthesis of biocytinyl peptides.¹

Biocytin has been used as an intracellular labeling reagent for neurons.²⁻¹² Advantages of using biocytin include its high solubility in aqueous solutions and small molecular weight, which facilitate its injection using micropipettes. Lucifer yellow, a fluorescent dye used for labeling neurons, can clog microelectrodes more readily than biocytin.² Horseradish peroxidase is also used as an intracellular marker; however, broken or beveled microelectrodes tips are needed to avoid clogging. Biocytin can be injected from non-beveled microelectrode tips,² injected by pressure or injected by iontophoresis.

Biocytin can be transported by neurons. Anterograde transport is predominant in rats,^{3,4} while both retrograde and anterograde transport occurs in primates.⁵ There are advantages of biocytin over other intracellular labeling reagents, such as *Phaseolus vulgaris* lectin (PHA-L). PHA-L can be used as a marker to reveal the fine detail of axonal and dendritic processes; however, it is expensive compared to biocytin and can be used only in certain animal species.⁵ Lucifer yellow labeling can be observed only through its fluorescent response, is prone to fading and cannot be used to provide a permanent record.²

Avidin or streptavidin conjugates can be used to incorporate biocytin. Conjugates with alkaline phosphatase, horseradish peroxidase, colloidal gold, fluorescein, rhodamine, and Texas Red[®] have been used.^{2,3} Therefore, detection can be achieved at the light, fluorescence, or electron microscope level. Biocytin can also be used in conjunction with histochemical staining procedures.⁵ Biocytin has also been used with rhodamine-labeled latex microspheres in a double-labeling application.⁶

Neuron Labeling Procedure

Note: The following protocol is an example application for this product. Specific applications will require optimization.

A. Additional Materials Required

- Lucifer yellow CH, lithium salt
- Wash Buffer: Phosphate-buffered saline (Product No. 28372, 0.1M phosphate, 0.15M sodium chloride; pH 7.2) with 0.5% Triton[®] X-100 Detergent (Product No. 28314)
- Streptavidin, Horseradish Peroxidase Conjugated (Product No. 21126) diluted to 25µg/mL in wash buffer
- Fixative: 5% sucrose, 0.1M potassium phosphate, pH 7.4, containing 4% paraformaldehyde
- Thermo Scientific DAB Substrate Kit (Product No. 34002) or Metal Enhanced DAB Substrate Kit (Product No. 34065)

B. Procedure

1. Fill microelectrode with 0.5M KCl containing 5% biocytin and 1% Lucifer yellow CH. Lucifer Yellow fluoresces with excitation of 355-425nm, which allows electrode tip visualization and confirmation of penetration of the target cell.¹³
2. Impale a single cell and characterize electrophysiologically.
3. Fill the neuron with biocytin using pulses of hyperpolarizing current (0.1-1.0nA for 100-500 milliseconds at 1Hz) for 10-20 minutes.
Note: Depolarizing current can also be used.²
4. Equilibrate for 30-60 minutes.
5. Incubate tissue overnight in fixative.
6. Rinse tissue with wash buffer.
7. Incubate tissue in Streptavidin-HRP solution. For sections with thickness of 75-150µm, incubate for 2 hours. For slices with thickness of 500µm, incubate for 24 hours.
8. Rinse tissue 5 × 20 minutes with wash buffer. Incubate tissue for 15 minutes in DAB substrate solution.
9. Rinse tissue with wash buffer. Mount tissue on slides coated with gelatin. Dehydrate tissue through a sequential graded ethanol series and clear with xylene.

Cited References

1. Bodansky, M. and Fagan, D.T. (1977). Synthesis of biocytin-containing peptides. *J Am Chem Soc* **99**:235-9.
2. Horikawa, K. and Armstrong, W.E. (1988). A versatile means of intracellular labeling: injection of biocytin and its detection with avidin conjugates. *J Neurosci Meth* **25**:1-11.
3. King, M.A., *et al.* (1989). Biocytin: a versatile anterograde neuroanatomical tract-tracing alternative. *Brain Res* **497**:361-7.
4. Izzo, P.N. (1991). A note on the use of biocytin in anterograde tracing studies in the central nervous system: application at both light and electron microscopic level. *J Neurosci Meth* **36**:155-66.
5. Lachica, E.A., *et al.* (1991). Morphological details of primate axons and dendrites revealed by extracellular injection of biocytin: an economic and reliable alternative to PHA-L. *Brain Res* **564**:1-11.
6. Tseng, G.-F., *et al.* (1991). Double-labeling with rhodamine beads and biocytin: a technique for studying corticospinal and other projection neurons *in vitro*. *J Neurosci Meth* **37**:121-31.
7. Deitch, J.S., *et al.* (1990). Confocal scanning laser microscope images of hippocampal neurons intracellularly labeled with biocytin. *J Neurosci Meth* **33**:61-76.
8. Vaney, D.I. (1991). Many diverse types of retinal neurons show tracer coupling when injected with biocytin or Neurobiotin. *Neurosci Letts* **125**:187-90.
9. Kawaguchi, Y., *et al.* (1990). Projection subtypes of rat neostriatal matrix cells revealed by intracellular injection of biocytin. *J Neurosci* **10**:3421-38.
10. Turner, J.N., *et al.* (1991). Confocal microscopy and three-dimensional reconstruction of electrophysiologically identified neurons in thick brain slices. *J Elec Microsc Tech* **18**:11-23.
11. Rønnekleiv, O.K., *et al.* (1990). A method for immunocytochemical identification of biocytin-labeled neurons following intracellular recording. *Biotechniques* **9**:432-8.
12. Granata, A.R. and Kitai, S.T. (1992). Intracellular analysis in vivo of different barosensitive bulbospinal neurons in the rat rostral ventrolateral medulla. *J Neurosci* **12**:1-20.
13. Vaney, D.I. (1991). Many diverse types of retinal neurons show tracer coupling when injected with biocytin or neurobiotin. *Neurosci Letts* **125**:187-90.

Triton is a trademark of The Dow Chemical Company. Texas Red is a trademark of Life Technologies Corp.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at www.thermoscientific.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

© 2011 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.