INSTRUCTIONS

EZ-Link® Biocytin

28022

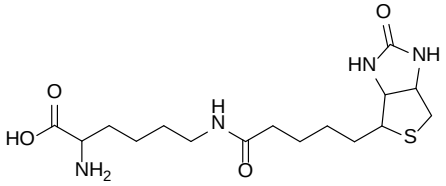
0450.3

Number	Description
28022	**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 ionotophoresis.

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

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