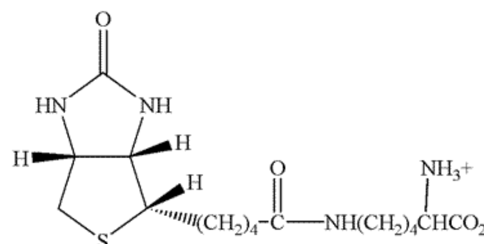
Biocytin

Very useful cellular tracer that can be introduced into cells by microinjection

Name :	**Biocytin** ε-biotinoyl-L-Lysine
Catalog Number :	FP-61959A, 100mg
CAS :	576-19-2
Structure :	$C_{16}H_{28}N_4O_4S$
Molecular Weight :	MW: 372.48
Soluble in:	Water or DMSO
Storage:	Room temperature (+4°C for long term) Protect from light and moisture



Introduction

- **Biocytin**, an a biotin conjugated to the epsilon amine of lysine, is a naturally occurring molecule in serum and urine. It serves to transport and recycle biotin through the body. Biocytin mimicks biotin effects [0] and is more polar than biotin.
- **Applications**: biocytin is used :
  -as an intermediate in the synthesis of biocytinyl peptides [1]. for detection applications thanks to its high binding affinity for avidin and streptavidin.
  -an intracellular labeling reagent for neurons, showing several advantage over other intracellular labeling reagents, as flexibility, easy microinjection [2-12] . It is first introduced into cells, fixed with aldelhyde-based fixatives and then detected with conjugates of avidin and streptavidin.
- Its high solubility in aqueous solutions and small molecular weight facilitate its injection using micropipettes compared with Lucifer yellow (a fluorescent dye that has been used for labeling neurons, that can clog microelectrodes much more readily) [2] , as well compared with direct injection of horseradish peroxidase (used as an intracellular marker, that also can clog) [2] . Biocytin also can be injected by pressure or ionotophoretically. It is also flexible for the choice of label, and can avoir for example fading problems encountered with Lucifer yellow [2] .
- Biocytin can be transported by neurons. Anterograde transport is predominant in rats [3,4]. Both retrograde and anterograde transport occurs in primates [5] . Additionnally, biocytin is less expensive and more flexible that the intracellular labeling reagent *Phaseolus vulgaris* lectin (PHA-L), used as a marker to reveal the fine detail of axonal and dendritic processes but that can be used in only certain species [5] .
- **Coupling to carboxyl** containing molecules require the use of glutaraldehyde (for in-situ fixation, IHC/IF), of of EDC (EDAC #UP52005) for labeling applications . Protocols are given in EDC technical sheet, in the literature, or you may set up your own protocol using following guidelines: The protein may be prepared in PBS or MES 0.1M pH5-6, and incubated for 2H at room temperature at 80µM with 2.8nM of Biocytin and 6.9mM of EDAC. Polymerization of the protein (and correlated precipitate) may occur, and reduced by decreasing EDAC ratio to protein or increasing biotin ratio. After desalting, the biotinylated protein may be analysed for biotin content (HABA assay #UP05361).
- **Detection**: Biocytin can be used with a variety of labels. Therefore, detection can be achieved by light absorbance, fluorescence, or electron microscope level in enzyme assays or cell assays. Conjugates with alkaline phosphatase, horseradish peroxidase, colloidal gold, fluorescein, rhodamine, and SulfoRhodamine101 (TR) have been used in several techniques 2,3, including histochemical staining procedures.5 .Chromogenic substrates can be chosen for use with the enzymes to yield insoluble colored precipitates. Even double labeling can be achived, i.e. with rhodamine-labeled latex microspheres 6

Guidelines for use

1- Transfer fixed slice into 10% Sucrose in 0.1 M Phosphate Buffer pH 7.6 for 2 hours. Transfer slice into 30% Sucrose in 0.1M Phosphate Buffer pH 7.6 overnight.
2- Cut slice and transfer sections into 0.1M Phosphate Buffer pH 7.6. Wash with phosphate buffer 3 x 3 mins.
3- Transfer sections in the following solution : 20 ml of 0.1M phosphate buffer, 200 µl of Triton X-100 and 40 µl of Streptavidin HRP. Incubate for 2 hours at room temperature.
4- Wash section in phosphate buffer 3 mins three times.
5- Incubate sections in DAB solution at room temperature for 20 mins.
Note : DAB solution is composed of DAB at 1mg/ml in water with 20 ml of 0.1M phosphate buffer pH7.6 and 120 µl.
6- To each incubating well add 10µl of 0.03% Hydrogen Peroxide every minute for 10 - 20 minutes.
7- Wash sections with phosphate buffer 5 mins three times.
8- Mount sections onto gelatine coated slide and allow to air dry.
9- Rinse sections with distilled water, then dehydrate, clear and mount coverslip.

References – Biocytin #FP6159-61959A

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Other information

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D-Biotin (FP-10685L) and carboxylated derivatives (PEO spacer…)
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Biocytin hydrazide (FP-22772A)
DAB, tablets (732311)

FT-61959A

EDC (UP52005; crosslinker for amine to carboxyl conjugations)
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