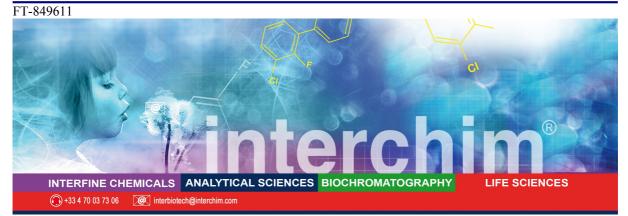
FluoProbes



Fluorescent & Biotinylated Cadaverines

Cadaverines (aminated biotins and fluorophores) are used as **polar tracer** in cell biology studies. They are also useful **building block** for biotin derivatives synthesis or conjugations to biotinylate carboxyls (DNA).

Biotin Cadaverines	cat.#	MW
Biotin-Cadaverine (N-(5-aminopentyl)biotinamide)	FP-849611, 25mg K	328.48
Spacer length: 18.9Angstroms HN NH CE ₂ CO ₂		
Spacer length: 18.9Angstroms Biotin-Cadaverine , TFA salt N-(5-aminopentyl)biotinamide, trifluoroacetic CAS:288259-39-2 $HN^{-}NH$ $CF_3CO_2^{-}$	FP-85594A, 25mg	442.5
Biotin-X-Cadaverine 5-(((N-(Biotinoyl)Amino)Hexanoyl)Amino)Pentylamine, free acid	FP-FI8951 , 20mg K	441.64
Biotin-lc-Cadaverine , TF 5-(((N-(Biotinoyl)Amino) Hexanoyl)Amino)Pentylamine, TFA salt CF ₃ CO ₂ H H H S H H NH H S H H NH H NH NH NH NH NH NH NH NH NH NH	FP-83882A, 20mg K	555.67
Biotin ethylenediamine, free acidSee analogs / thiolsSoluble in DMSOreactive biotin tracers	FP-AM544A, 25mg K	367.32
Lucifer Yellow Cadaverine Biotin-X , dipotassium salt MW: 873.13 $\lambda_{ex}\lambda_{em}=428/532$ nm. Yellow solid soluble in water	FP-M1210A, 10mg M	873.1 2K* CH ₂)5-N
Biocytin ε-Biotinoyl-L-Lysine; ε-N-[d-biotinyl]-L-lysine	FP-61959A, 100mg (Z)	372.48
Water soluble polar tracer More information in tech sheet $\# 61959A$		

LIFE SCIENCES

terchi

211 BP Fax

FluoProbes

Fluorescent Cadaverines	cat.#	MW
	FP-46576B, 25mg M	653.4
5-FITC cadaverine, 2HCl salt	11 100 / 0D, 20 mg	
ROX cadaverine (mixed isomer)	FP-BV3800, 10mg M	862.8
5(6)-ROX cadaverine Fluorescein cadaverine, TFA salt	FP-CE8760, 25mg	719.6
λ_{ex} (λ_{em} (pH9) = 493/517 nm Soluble in DMSO, DMF, or pH>6.5 buffers		
5-FAM cadaverine	FP-AM846A, 10mg M	460.5
$\lambda_{ex} \setminus \lambda_{em}(pH > 7.0) = 494/521 \text{ nm}$ Soluble in DMSO, DMF	FP-AM846B, 100mg	
5(6)-FAM cadaverine	FP-JQ4290, 1 g	460.5
$\lambda_{ex} = \frac{1}{2} \lambda_{em}(pH > 7.0) = 494/521 \text{ nm}$ Soluble in DMSO, DMF		
5-FITC cadaverine, 2HCl salt	FP-46576B, 25mg M	653.4
Fluorescein cadaverine, diBr salt	FP-CE9030 , 5mg	564.5
TMR cadaverine Soluble in DMSO or DMF	FP-60053A, 10mg	514.62
$\lambda_{ex} \setminus \lambda_{em}(MeOH) = 544/571 nm$		
Sulforhodamine 101 cadaverine	FP-M1206A, 5mg	690.9
$\lambda_{ex} (\lambda_{em} (MeOH) = 583/603 \text{ nm}$ Soluble in DMSO or DMF		
Special Cadaverines	cat.#	MW
Biotin ethylenediamine, hydrobromide	FP-76658A, 25mg K	367.32
(equivalent to Neurobiotin [™])		
Soluble in DMSO		
Useful anterograde and transneuronal tracer		
Building block to biotinylate carboxyls	S	
Biotin ethylenediamine, iodoacetamide	FP-AM547A, 25mg	454.33

Biotin ethylenediamine, iodoacetamide Soluble in DMSO

TS Biotin-ethylenediamine, sodium salt	FP-JW7170, 25mg M	462.54	
Soluble in DMSO or water Useful for labeling thiols exposed out-sided on membranes		0 ₩ NH-CCH₂S-	0 S−O*Na* S−O*Na* 0
TS Biotin-XX-ethylenediamine, sodium salt The extended spacer version of product #FP-JW717	FP-JW7190, 25mg M	688.86	
HN H H H H H H H H H H H H H H H H H H			

FP-86413A, 25mg Lucifer Yellow Cadaverine Soluble in DMSO and H2O $\lambda_{\text{ex}} \ \lambda_{\text{em}} = 425 \ nm / \ 531 \ nm$ Water soluble polar tracer - More information in tech sheet #15437A

211 BP Fax

Storage: $+4^{\circ}C$ (K); or $-20^{\circ}C$ ($+4^{\circ}C$ for short term) (M)

LIFE SCIENCES

"O3S

М

(CH₂)₅NH₂

2K*

SO3 NH2

533.7

FT-849611

Applications – Biotin cadaverins:

- Biotinylation of Carboxylic Acids containing biomolecules (<u>alHakim1988</u>), notably DNA and peptides.
- Transglutaminase-mediated modification of glutamine residues in cells and certain proteins (Lai 1994, Slaughter1992)
- Colorimetric assay of blood coagulation factor XIII in plasma (Lee 1988)
- > Polar tracing (anterograde and transneuronal tracer)

Scientific information

Cadaverines (aminated derivatives) can be coupled to activated carboxy groups with EDC (<u>UP52005</u>) or to sulfonyl chlorides. They are conjugated notably to nucleic acids, to protein in in-situ cells, and other applications such as enzyme assays.

Cadaverines are conjugated to fluorescent and biotin labels for use in detection techniques such as immunoassays, interaction studies, cell microscopy or flow cytometry

Biotin cadaverines are notably used as building blocks to biotinylated DNAs, proteins. Product #FP855594A is used in colorimetric assays for factor XII carbonyls and for cellular transglutaminases. It exists also with a long chain spacer (FP-FI8951), and as a free base form (#FP-849611 and FP-83882A). (BiotinAmido)PentylAmine is relatively water soluble (up 50nM in water) and contains a terminal primary amine, separated from biotin by a 18.9Angstroms long spacer.

Biotin ethylenediamine, besides biotinylation applications, is notably useful as an anterograde and transneuronal tracer. It is available as several salts (see related products). It is available with several thiol reactive substituants:

- The Hydrobromide and Iodoacetamide groups are standard thiols reactive. **Biotin ethylenediamine** hydrobromide was popularized as NeuroBiotin[™]. Compared to biocytin and other neuronal labels, it is more soluble, iontophoreses better, remains in cells longer, is not toxic, and can be fixed in place with formalin or glutaraldehyde. Use in gap junctional coupling has also been reported. See a protocol of use in Neurons below.
- The TS (thiosulfate) group of **TS biotin ethylenediamine** is a highly selective thiol labeling probe. Similar to the MTS group, it reacting with thiols at nearly neutral pH, however, unlike the neutral MTS group, it is negatively charged and thus probes bearing a TS group are ideal for specifically labeling thiols exposed at the exterior cell surface because charged probes are less likely to enter cells. The XX spacer of product #FP-JW7190 should facilitate the interaction between biotin and avidin or streptavidin.
- **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 is used -as an intermediate in the synthesis of biocytinyl peptides (Bodansky 1977). 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 (Rønnekleiv 1990). It is first introduced into cells, fixed with aldelhyde-based fixatives and then detected with conjugates of avidin and streptavidin. See technical sheet #61959A for more information.

- Fluorescent cadaverines are used for labeling purposes, enzymes assays.
- **Coupling to carboxyl** containing molecules require the use of glutaraldehyde (for in-situ fixation, IHC/IF), or of EDC (EDAC #<u>UP52005</u>) for labeling applications. See below.

Directions for use

<u>Protocol</u> of Intracellular labeling of Neurons with Biotin-EthyleneDiamine (source: <u>Kita</u> <u>1991</u>)

A. Intracellular recording electrode:

LIFE SCIENCES

Glass micropipetes were pulled from capillaries (1-2 mm, O.D.) containing a microfilament. They were filled with 2% Biotin-EthyleneDiamine 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 MW.

FT-849611

B. Intracellular injection:

Neurons in in vitro brain slice preparations or anesthetized animals were impaled with the recording electrodes and were injected with Biotin-EthyleneDiamine 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 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.

Protocol of Coupling to carboxyls

Coupling to carboxyl containing molecules require the use of glutaraldehyde (for in-situ fixation, IHC/IF), or of EDC (EDAC #<u>UP52005</u>) 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 80μ M with 2.8nM of BiotinAmidoPentylAmine 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 analyzed for biotin content (HABA assay).

References - Biotin-cadaverines

al-Hakim AH, Hull; R Biochem J 251, 935-938 (1988); Chemically synthesized non-radioactive biotinylated long-chain nucleic acid hybridization probes; PN7428. [85594A]

Kawaguchi T, Decho AW. Anal Biochem 304, 266-267 (2002); In situ analysis of carboxyl and sulfhydryl groups of extracellular polymeric secretions by confocal laser scanning microscopy." [85594A]

Moaddel M, Falls LA, Farrell DH. J Biol Chem 275, 32135-32140 (2000). The role of gamma A/gamma ' fibrinogen in plasma factor XIII activation." [85594A]

Lai TS, Achyuthan KE, Santiago MA, Greenberg GS J Biol Chem 269, 24596-24601 (1994); Carboxyl-terminal truncation of recombinant factor XIII A-chains. Characterization of minimum structural requirement for transglutaminase activity; PN17717. [85594A]

Lee K.Y., Birckbichler, P.J. and Patterson, M.K., Jr. Clin. Chem. 34, 906-910.(1988); Colorimetric assay of blood coagulation factor XIII in plasma.

Slaughter TF, Achyuthan KE, Lai TS, Greenberg CS.; Anal Biochem 205, 166-171 (1992) PN14664; A microtiter plate transglutaminase assay utilizing 5-(biotinamido)pentylamine as substrate. [85594A]

1/ J. Histochem. Cytochem. 38, 377(1990); 2) Annals NY Acad. Sci. 463, 214(1984); 3) Biochem. J. 251, 935(1988), [83882A]

References – Biocytin #FP-61959A

IEE SCIENCES

Bodansky M. and Fagan, D.T. (1977); J. Am. Chem. Soc. 99, 235-9. Synthesis of biocytin-containing peptides.

Rønnekleiv, O.K., et al. (1990). Biotechniques 9, 432-8. A method for immunocytochemical identification of biocytin-labeled neurons following intracellular recording.

References – Biotin-Ethylene DiAmine #76658A

Perez-Orive J, Mazor O, Turner GC, Cassenaer S, Wilson RI, Laurent G.; Science 297, 359-365 (2002); Oscillations and sparsening of odor representations in the mushroom body.

Personius KE, Balice-Gordon RJ. Neuron 31, 395-408 (2001); Loss of correlated motor neuron activity during synaptic competition at developing neuromuscular synapses.

Kita H., and Armstrong, W. ; J. Neurol. Sci. 37, 141, (1991); A biotin-containing compound N-(2-aminoethyl)biotinamide for intracellular labeling and neuronal tracing studies: comparison with biocytin.

FT-849611

References - Lucifer Yellow Cadaverine

Giordano L, Jovin TM, Irie M, Jares-Erijman EA. J Am Chem Soc 124, 7481-7489 (2002) Pantoja S, Lee C, Marecek JF, Palenik BP. Anal Biochem 211, 210-218 (1993)

Related documents and products

See <u>BioSciences Innovations catalogue</u> and <u>e-search tool</u>.

<u>#10685A</u>	D-Biotin (<u>UP10685</u>) and carboxylated derivatives (PEO spacer)
<u>#61959A</u>	Biocytin (ɛ-biotin-lysine)
<u>#84961A</u>	Aminated biotins (including shorter and PEO spacers versions)
<u>#39375A</u>	IminoBiotin (UP39375) and NHS-IminoBiotin (UP35329)
<u>#52117A</u>	Amine activated Biotins (i.e NHS-Biotin UP52117)
<u>#52005A</u>	EDC (<u>UP52005</u> ; crosslinker for amine to carboxyl conjugations)
<u>#05361D</u>	HABA (<u>UP05361</u> ; biotin quantitation)
<u>#15437A</u>	Lucifer Yellow probes
<u>FPstd</u>	FluoProbes labels (alternative labels with improved features) – amine derivatives

Product for R&D use only

Neurobiotin is a trademark from Vector Labs.

For any information, please ask FluoProbes / Interchim

Rev.H11E