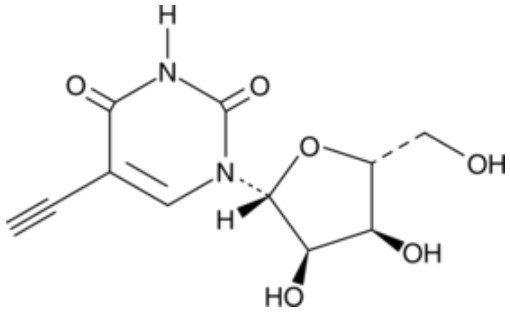
Product Data Sheet

Product Name: 5-Ethynyluridine
Cat. No.: GC49245

Chemical Properties

Cas. No.	69075-42-9		
SMILES	O[C@H]1[C@@](N2C=C(C(NC2=O)=O)C#C)([H])O[C@@H]([C@H]1O)CO		
Formula	C11H12N2O6	M.Wt	268.2
Solubility	DMF: 10 mg/ml,DMSO: 10 mg/ml,PBS (pH 7.2): 5 mg/ml	Storage	-20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		

Structure



Protocol

Labeling of Cultured Cells and Mouse Tissues with 5-Ethynyluridine [1]

This protocol was compiled from the original study. This protocol is for reference only. Please adjust according to actual needs.

1. Cell Labeling

(1) Cell Culture and Reagent Preparation: Cells are cultured on glass coverslips in DMEM (or other basic media) supplemented with 10% calf serum, penicillin, and streptomycin. 5-Ethynyluridine is dissolved in DMSO to prepare a 100mM stock solution. Cell experiments use 1% DMSO as control.

(2) Cell Labeling: 5-Ethynyluridine is added to complete culture medium. Cells are incubated for 10-24h.

(3) Cell Washing and Fixation: Cells are fixed at room temperature for 30min with 125mM Pipes pH=6.8, 10mM EGTA, 1mM magnesium chloride, 0.2% Triton X-100, and 3.7% formaldehyde.

(4) Click Chemistry Detection: Fixed cells are rinsed with TBS. Click staining is performed at room temperature for 30min. The staining solution contains 100mM Tris pH=8.5, 1mM $CuSO_4$, 10-50μM fluorescent azide (Alexa594-azide, Alexa568-azide, or TMR-azide), and 100mM ascorbic acid. After staining, cells are washed multiple times with TBS containing 0.5% Triton X-100. Hoechst is used for counterstaining. Fluorescence microscopy is used for observation.

Mouse Labeling

(1) Animal Labeling: Three-week-old mice receive intraperitoneal injection of 100μL 20mg/mL 5-Ethynyluridine in PBS (2mg per mouse). Tissues are collected 5h later. Uninjected littermates serve as negative controls.

(2) Tissue Processing and Section Staining: Small intestine, kidney, liver, and spleen are fixed in formalin overnight. Tissues are embedded in paraffin and sectioned. Sections are deparaffinized and rehydrated. Sections are stained with 25μM TMR-azide for 30min using the cell click method. Hoechst is used for counterstaining. Fluorescence microscopy is used for observation.

Ex Vivo Organ Staining

(1) Whole-Mount Organ Staining: One-centimeter-long small intestine from 5-Ethynyluridine-injected mice is fixed. The intestine is cut open longitudinally. The tissue is stained with 50μM TMR-azide for 1h. The tissue is washed by end-over-end rotation in 20mM Hepes pH=7.5, 500mM NaCl, and 1% Triton X-100 for approximately 24h until background is very low. OliGreen is used to stain DNA. A fluorescent dissecting microscope is used for imaging.

Precautions:

(1) 5-Ethynyluridine is incorporated into transcripts produced by RNA polymerases I, II, and III. 5-Ethynyluridine does not significantly label DNA. Nucleolar signal is the strongest.

(2) Click reaction requires Cu(I) catalysis. Ascorbic acid is added immediately before use.

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Fluorescent azides require light-protected operation.

(3) For your safety and health, please wear a lab coat and disposable gloves during operation.

References:
[1] Jao CY, Salic A. Exploring RNA transcription and turnover in vivo by using click chemistry. Proc Natl Acad Sci U S A. 2008 Oct 14;105(41):15779-84.

Background

5-Ethynyluridine is a cell-permeable 5-ethynyl uridine analog. 5-Ethynyluridine reacts with azide probes via copper-catalyzed azide-alkyne cycloaddition click chemistry to label, isolate, and sequence newly synthesized RNA. 5-Ethynyluridine serves as a low-immunogenic uridine variant for mRNA modification research. 5-Ethynyluridine is useful for studies on transcription dynamics, RNA metabolism, transcriptional changes in neurological diseases, and mRNA vaccines and therapeutics[1-4].

References:
[1] Maassen S, Coenen B, Dulk S, et al. 5-Ethynyluridine: A Bio-orthogonal Uridine Variant for mRNA-Based Therapies and Vaccines. Chembiochem. 2023 Mar 1;24(5):e202200658.
[2] Neitz H, Höbartner C. A tolane-modified 5-ethynyluridine as a universal and fluorogenic photochemical DNA crosslinker. Chem Commun (Camb). 2023 Oct 5;59(80):12003-12006.
[3] Qu D, Zhou L, Wang W, et al. 5-Ethynylcytidine as a new agent for detecting RNA synthesis in live cells by "click" chemistry. Anal Biochem. 2013 Mar 1;434(1):128-35.
[4] Ierusalimsky VN, Balaban PM. Long-living RNA in the CNS of terrestrial snail. RNA Biol. 2018 Feb 1;15(2):207-213.

Caution: Product has not been fully validated for medical applications. For research use only.
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