5-ethynyl uridine (EU)

Catalog no. E10345

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
5-ethynyl uridine (EU)	5 mg	• ≤–20°C • Desiccate	When stored as directed, the product is stable for at least 1 year

Introduction

5-Ethynyl uridine (EU) is a novel alternative for bromo uridine (BrU or BrUTP)[1] to directly image spatially and temporally nascent global RNA transcription both *in vitro* and *in vivo*. EU is a nucleoside analog of uracil, and is incorporated into RNA during active RNA synthesis. Detection of the incorporated EU is based on a powerful click chemical reaction that utilizes bio-orthogonal or biologically unique moieties, specifically azides and alkynes.[2-5]

EU contains an alkyne that can react with an azide-containing detection reagent in the Click-iT® Cell Reaction Buffer Kit (Cat. no. C10269) to form a stable triazole ring (Figure 1, Table 2). Because click chemistry-based label and detection tags react selectively and specifically with one another, you can apply click-labeled molecules to complex biological samples and subsequently detect with unprecedented sensitivity due to an extremely low background.

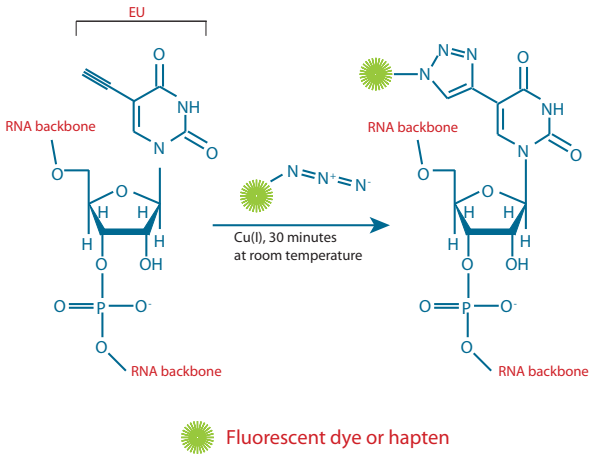

Figure 1. Click reaction between EU and azide-modified dye or hapten.

Table 2. Azide-modified fluorophores and haptens for detection.

Label	Ex/Em*	Cat. no.	Use	Detection platform
Alexa Fluor® 488	495/519	A10266	Fluorescent dye or hapten	Flow, HCS, Fluorescence microscopy
Alexa Fluor® 594	590/617	A10270	Fluorescent dye	HCS, Fluorescence microscopy
Alexa Fluor® 647	650/655	A10277	Fluorescent dye	Flow, HCS, Fluorescence microscopy
Biotin**	NA	B10184	Hapten	Flow, HCS, Fluorescence microscopy,
Oregon Green® 488	496/524	O10180	Fluorescent dye or hapten	Flow, HCS, Fluorescence microscopy
Tetramethylrhodamine (TAMRA)	555/580	T10182	Fluorescent dye or hapten	HCS, Fluorescence microscopy

*Approximate fluorescence excitation (Ex) and emission (Em) maxima, in nm. **Requires streptavidin.

Click chemistry fills the void when methods such as direct labeling with biotin or a fluorophore (MW ~300–1,000 daltons), or the use of antibodies (MW ~150,000 daltons) are not applicable or efficient, particularly with small molecules and complex samples (Figure 2). The click chemistry label is small enough that the tagged molecules (*e.g.*, nucleotides, sugars, and amino acids) are acceptable substrates for the enzymes that assemble these building blocks into biopolymers.

It has been demonstrated that EU can be efficiently incorporated into RNA by several polymerases, but it does not incorporate into DNA.[6] The click detection reaction requires copper as the catalyst, and copper has been shown to induce some RNA degradation (scission). Because of this, we do not recommend RNA sequencing following the click reaction at this time. However, there are other novel applications for EU. For example, the small size of the detection reagent enables the simultaneous detection of RNA-interactive proteins with antibodies, which cannot be accomplished if antibodies are used for the detection of BrU.

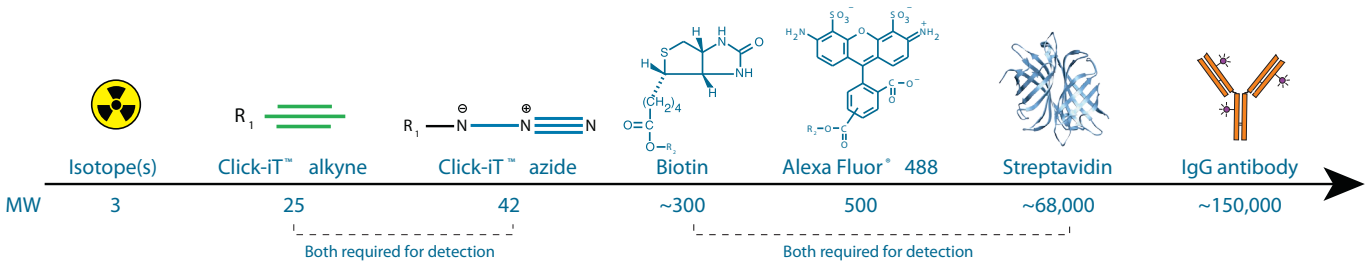

Figure 2. Relative size of detection molecules commonly used in cellular analysis.

Before You Begin

Preparing Stock Solution

EU is readily soluble in DMSO, alcohol, water, or aqueous buffers. For use with *in vitro* applications, we recommend preparing a 0.1–1 M EU stock solution in DMSO or an aqueous buffer. You may store any unused EU stock solution at ≤–20°C for up to one year.

Handling and Disposal

EU is a nucleoside analog which can be incorporated into RNA. Handle and disopose of EU in compliance with all pertaining local regulations. When EU is dissolved in DMSO, which is known to facilitate the entry of organic molecules into tissue, use additional precautions appropriate for the hazards posed by such materials.

Experimental Protocols

EU Labeling

In initial experiments, we recommend testing a range of EU concentrations to determine the optimal concentration. If currently using a BrU-based assay, a similar concentration and duration to BrU is a good starting concentration for EU. The optimal concentration may vary depending upon the duration of the pulse, with lower concentrations recommended for longer incubations. With cultured cells, acceptable EU incorporation has been observed with 0.5–5 mM EU for 0.5–24 hours.

Note: Do not exceed DMSO concentrations of 0.5% when labeling live cells.

EU Detection

You may detect incorporated EU with an available dye- or hapten-containing azide (Table 2) together with the Click-iT® Cell Reaction Buffer Kit (Cat. no. C10269). If signal amplification is desired, in addition to the classic hapten, biotin, you may use three of the fluorescent dyes, Alexa Fluor® 488, Oregon Green® 488, and tetramethylrhodamine (TAMRA), with their corresponding anti-dye antibodies.

Imaging and Analysis

See Table 2 for the appropriate fluorescence excitation/emission maxima for detection of EU following the click reaction.

References

1. RNA 6, 1750 (2000); **2.** ChemBioChem 4, 1147 (2003); **3.** J Am Chem Soc 125, 3192 (2003); **4.** Angew Chem Int Ed Engl 41, 2596 (2002); **5.** Angew Chem Int Ed Engl 40, 2004 (2001); **6.** PNAS 105, 15779 (2008).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
E10345	5-ethynyl uridine (EU)	5 mg
Related Products		
A889	anti-fluorescein/Oregon Green®, rabbit IgG fraction *1 mg/mL*	0.5 mL
A982	anti-fluorescein/Oregon Green®, rabbit IgG fraction, biotin-XX conjugate *1 mg/mL*	0.5 mL
A6397	anti-tetramethylrhodamine, rabbit IgG fraction *1 mg/mL*	0.5 mL
A6413	anti-fluorescein/Oregon Green®, rabbit IgG Fab fragment *0.5 mg/mL*	0.5 mL
A6421	anti-fluorescein/Oregon Green®, mouse IgG_{2a}, monoclonal 4-4-20	0.5 mg
A10266	Alexa Fluor® 488 azide (Alexa Fluor® 488 5-carboxamido-(6-azidohexanyl), bis(triethylammonium salt)) *5-isomer*	0.5 mg
A10270	Alexa Fluor® 594 azide (Alexa Fluor® 594 carboxamido-(6-azidohexanyl), triethylammonium salt)) *mixed isomers*	0.5 mg
A10277	Alexa Fluor® 647 azide, triethylammonium salt	0.5 mg
A11090	anti-fluorescein/Oregon Green®, rabbit IgG fraction, Alexa Fluor® 488 conjugate *1 mg/mL*	0.5 mL
A11091	anti-fluorescein/Oregon Green®, rabbit IgG fraction, Alexa Fluor® 594 conjugate *1 mg/mL*	0.5 mL
A11094	anti-Alexa Fluor® 488, rabbit IgG fraction *1 mg/mL*	0.5 mL
A11095	anti-fluorescein/Oregon Green®, goat IgG fraction *1 mg/mL*	0.5 mL
A11096	anti-fluorescein/Oregon Green®, goat IgG fraction, Alexa Fluor® 488 conjugate *1 mg/mL*	0.5 mL
A21250	anti-fluorescein/Oregon Green®, rabbit IgG fraction, R-phycoerythrin conjugate *2 mg/mL*	250 µL
A21253	anti-fluorescein/Oregon Green®, rabbit IgG fraction, horseradish peroxidase conjugate	0.5 mg
B10184	biotin azide (PEG_4 carboxamide-6-azidohexanyl biotin)	1 mg
C10269	Click-iT® Cell Reaction Buffer Kit	1 kit
O10180	Oregon Green® 488 azide (Oregon Green® 488 6-carboxamido-(6-azidohexanyl), triethylammonium salt) *6-isomer*	0.5 mg
Q10137MP	Qdot® 565 goat anti-fluorescein conjugate *2 µM* *whole IgG*	500 µL
Q15421MP	Qdot® 655 goat anti-fluorescein conjugate *1 µM* *whole IgG*	200 µL
Q15431MP	Qdot® 565 goat anti-fluorescein conjugate *1 µM* *whole IgG*	200 µL
T10182	tetramethylrhodamine (TAMRA) azide (tetramethylrhodamine 5-carboxamido-(6-azidohexanyl)) *5-isomer*	0.5 mg

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