Jun 30, 2025 Version 2

5-ethynyl uridine labeling of nascent mitochondrial genome transcription V.2

DOI
https://dx.doi.org/10.17504/protocols.io.261gero6wl47/v2

Tejashree Waingankar[1], John Smolka[1], Samantha C Lewis[1]

[1]UC Berkeley

S Samantha C Lewis
UC Berkeley

DOI: https://dx.doi.org/10.17504/protocols.io.261gero6wl47/v2

Protocol Citation: Tejashree Waingankar, John Smolka, Samantha C Lewis 2025. 5-ethynyl uridine labeling of nascent mitochondrial genome transcription. **protocols.io** https://dx.doi.org/10.17504/protocols.io.261gero6wl47/v2Version created by **Samantha C Lewis**

Manuscript citation:
Adam Begeman *et al.*, Spatial analysis of mitochondrial gene expression reveals dynamic translation hubs and remodeling in stress.*Sci. Adv.***11**,eads6830(2025).DOI:**10.1126/sciadv.ads6830**

Protocol status: Working
We use this protocol and it's working

Created: June 30, 2025

Last Modified: June 30, 2025

Protocol Integer ID: 221341

Keywords: Fluorescence microscopy, Transcription, mitochondria, mtDNA, click chemistry, fluorescent readout of localized mitochondrial gene transcription, mitochondrial genome transcription, localized mitochondrial gene transcription, mitochondrial genome transcription this protocol, ethynyl uridine labeling of nascent, immunofluorescence detection of mtdna, ethynyl uridine labeling, mtdna, proteins in cultured cell, mitochondrial genome, genome

Funders Acknowledgements:
Sloan Research Foundation
Grant ID: FG-2023-20384
NSF
Grant ID: 2339182

Abstract

This protocol details a method for 5-ethynyl uridine labeling of nascent mitochondrial genome transcription coupled with immunofluorescence detection of mtDNA-binding proteins in cultured cells. The result is a fluorescent readout of localized mitochondrial gene transcription compatible with high-resolution microscopy.

Guidelines

This protocol was developed to work with cultured IMR90 fibroblasts. It also works with cultured murine sensory neurons.

Materials

Buffers and reagents:

- 1 mM Triptolide diluted in DMSO
- 0.5 M 5-ethynyl uridine (EU) in DMSO
- Mitotracker Deep Red (MTDR)

  MitoTracker™ Deep Red FM - Special Packaging **Thermo Fisher Catalog #**M22426
- Click-iT™ Plus EdU Alexa Fluor™ 647 Imaging Kit **Thermo Fisher Catalog #**C10640
- **Permeabilization solution:** DPBS + 0.1% Triton X-100
- **Blocking buffer:** 0.1% TBST + 1% BSA
- **culture media:** Complete MEM: For 50 ml

	A	B
	Horse serum	5 ml
	Pen/strep antibiotic	500 µl
	L-Glutamine	500 µl
	MEM vitamin supplement	500 µl
	MEM	43.5 ml

Method

1d 14h 35m

1 Culture cells in vitro for 24:00:00 in 300 µL complete MEM on a coverslip or in a 35 mm glass bottom culture dish. 1d

2 Remove the 300 µL of culture media and add pre-warmed 1 mL of complete culture media.

3 Add 1 µL of [M] 1 millimolar (mM) Triptolide to the media and incubate at 37 °C for 01:00:00. 1h

4 Add 1 µL of [M] 0.5 Mass Percent EU to the media and incubate for 03:00:00 at 37 °C. 3h

5 After 2h, add [M] 50 nanomolar (nM) MTDR to the culture media.

6 Remove the media and replace it with fresh, complete culture media. Incubate at 37 °C for 00:10:00. 10m

7 Fix with pre-warmed 4% PFA for 00:20:00 at Room temperature. 20m

8 Add 200 µL of [M] 1 Mass Percent Glycine to quench the reaction for 00:05:00 at Room temperature. 5m

9 Add 2 mL of permeabilization solution for 00:10:00 at Room temperature. 10m

10 Replace the permeabilization solution with the blocking buffer for 00:10:00 at Room temperature. 10m

11 Replace the blocking buffer solution with primary antibody solution diluted in blocking buffer; incubate at 4 °C Overnight . 8h

12 Prepare a click reaction buffer mixture as described in the manufacturer's protocol.

13 Incubate at Room temperature for 00:30:00 while protected from light. 30m

14 Remove the solution completely and wash gently with 1 mL of blocking buffer.

15 Replace with freshly prepared secondary antibody solution diluted in blocking buffer; incubate for 01:00:00 at Room temperature , protected from light. 1h

16 Wash once with the blocking buffer for 00:10:00 . 10m

17 Cover cells in 500 µL DPBS and proceed with fluorescence microscopy imaging.

Protocol references

Adam Begeman *et al.*, Spatial analysis of mitochondrial gene expression reveals dynamic translation hubs and remodeling in stress.*Sci. Adv.***11**,eads6830(2025).DOI:**10.1126/sciadv.ads6830**

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. doi: 10.1073/pnas.0808480105. Epub 2008 Oct 7. PMID: 18840688; PMCID: PMC2572917.

Fantoni NZ, El-Sagheer AH, Brown T. A Hitchhiker's Guide to Click-Chemistry with Nucleic Acids. Chem Rev. 2021 Jun 23;121(12):7122-7154. doi: 10.1021/acs.chemrev.0c00928. Epub 2021 Jan 14. PMID: 33443411.