Nov 19, 2024

L-HPG Metabolic labeling of mitochondrial translation in cultured cell lines

DOI
https://dx.doi.org/10.17504/protocols.io.3byl4wedrvo5/v1

Adam Begeman[1], Samantha C Lewis[1]

[1]UC Berkeley

S Samantha C Lewis
UC Berkeley

DOI: https://dx.doi.org/10.17504/protocols.io.3byl4wedrvo5/v1

Protocol Citation: Adam Begeman, Samantha C Lewis 2024. L-HPG Metabolic labeling of mitochondrial translation in cultured cell lines. **protocols.io** https://dx.doi.org/10.17504/protocols.io.3byl4wedrvo5/v1

Protocol status: Working
This is our working protocol as of 11/18/2024.

Created: October 23, 2024

Last Modified: November 19, 2024

Protocol Integer ID: 110941

Keywords: L-HPG, Immunofluorescence, translation, mitochondria, mitoribosome, click chemistry, hpg metabolic labeling of mitochondrial translation, metabolic labeling of mitochondrial translation, hpg metabolic labeling, mitochondrial translation, metabolic labeling, cultured cell, homopropargylglycine, hpg, cell lines this protocol, cell line

Funders Acknowledgements:
NIH
Grant ID: R35GM147218
NSF
Grant ID: 2339182

Abstract

This protocol describes the metabolic labeling of mitochondrial translation in cultured cells (such as IMR90) using an amino acid L-Homopropargylglycine (L-HPG).

Guidelines

Protocol Notes:

- 15 min, 30 min, and 1 hr incubation times have been used
  5 minutes has been published
- Can click with either 647 or 488 azide
- This protocol is **NOT** compatible with glutaraldehyde fixation due to cytoplasmic signal drowning out mitochondrial

Materials

Buffers and Reagents:

- Fixative: 4% PFA in DPBS (lacking Calcium and Magnesium), pH 7.4
- Quenching Solution: 1M Glycine in DPBS
- Permeabilization Solution: DPBS + 0.1% Triton-X 100
- Blocking/ Staining Solution: TBST + 1% BSA
- DPBS
- Methionine Free DMEM (https://www.thermofisher.com/order/catalog/product/21013024)
- 50 mg/mL Cycloheximide (1000x)
- 50 mg/mL Chloramphenicol (1000x)
- L-HPG (50 mM) (1000x) (https://www.thermofisher.com/order/catalog/product/C10186?SID=srch-srp-C10186)
- Click Reaction Cocktail (**Made Fresh Right Before Use**)

Cultured cell line

- IMR90 fibroblast, ATCC #CCL-186 (https://www.atcc.org/products/ccl-186)

Note

Following volumes are for a single 35 mm imaging dish

	A	B
	Reaction buffer	440 uL
	Copper protectant solution	10 uL
	Fluorescent azide	1.2 uL
	Click reaction additive solution (1:10 solution in water of stock additive)	50 uL

Procedure

1h 57m

1 Remove media from adherent IMR90 cells cultured in 35 mm cell culture dish and wash once with DPBS pre-warmed to 37 °C .

2 Replace DPBS with 1 mL methionine free DMEM pre warmed to 37 °C .

3 Incubate at 37 °C for 00:10:00 . 10m

4 Add to cell culture dish Cycloheximide to a final concentration of 50 µg/ml (1:1000) e.g. 1 microliter into 1 mL of Methionine-free medium.

> Note
>
> Add Chloramphenicol to a final concentration of 50 µg per ml (1:1000) for mitochondrial translation inhibition control.

5 Incubate at 37 °C for 00:20:00 . 20m

6 Add L-HPG to dish to a final concentration of [M] 50 micromolar (µM) (1:1000 dilution into 1 mL) and incubate at 37 °C for desired amount of time.

7 Remove media and replace with methionine free DMEM pre warmed to 37 °C .

8 Incubate at 37 °C for 00:05:00 . 5m

9 Remove media from cell culture dish and immediately cover the dish with 2 mL fixative solution pre warmed to 37 °C .

10 Incubate at Room temperature for 00:30:00 . 30m

11 Quench fixation with 200 µL of quenching solution (00:02:00). 2m

12 Wash 1x with DPBS.

13 Remove DPBS and cover the dish with 2 mL of permeabilization solution and incubate at Room temperature for 00:20:00 . 20m

14 Wash 1X with DPBS.

15 Remove DPBS and add 500 µL click reaction cocktail and incubate at Room temperature in the dark for 00:30:00 . 30m

> Note
>
> Click reaction cocktail should be made fresh in the order above directly before use.

16 Add 1.5 mL blocking solution to dish and then aspirate off blocking solution/ click cocktail mixture.

17 At this point can move on to immunofluorescence.

Protocol references

Literature References:

1. https://doi.org/10.1073/pnas.2008778118
2. https://doi.org/10.15252/embr.202051635
3. https://rnajournal.cshlp.org/content/early/2022/03/07/rna.079097.122

Acknowledgements

We thank the Lewis lab for helpful discussion.