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

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Protocol status: Working

This is our working protocol as of 11/18/2024.

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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 $50\text{ }\mu\text{g/ml}$ (1:1000) e.g. 1 microliter into 1 mL of Methionine-free medium.



Note

Add Chloramphenicol to a final concentration of $50\text{ }\mu\text{g per ml}$ (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 $50\text{ }\mu\text{M}$ (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 μ L 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 μ L 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>

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