

Analysis of the Incorporation of Carbon Atoms from Radioactive Lactate into Proteins

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[Abstract] This method allows to analyze if the carbon atoms of lactate are embedded into proteins. Indeed, mammalian cells express the transporter of monocarboxylic acids (called MCT1) that allows the entry of lactate into the cell. To this end, cells are incubated for 24 h with the culture medium containing lactate uniformly labeled with carbon 14 and then, lactate inside the cell is evaluated by counting the radioactivity by a scintillator.

Materials and Reagents

1. [U-¹⁴C] lactate (U: uniformly labelled) (PerkinElmer, catalog number: NEC599250UC)
2. Phosphate buffered saline (pH 7) (Sigma-Aldrich, catalog number: D8537)
3. Trichloroacetic acid (Sigma-Aldrich, catalog number: T6399)
4. Liquid scintillation (PerkinElmer, catalog number: 6NE9529)
5. Vials for scintillator (PerkinElmer)
6. Dulbecco's Modified Eagle Medium (DMEM)
7. Eppendorf tube
8. Water
9. Cell scrapers (Euroclone)

Equipment

1. Incubator for cell culture
2. Petri dish for cell culture (six inches diameter)
3. Scintillator
4. Burk chamber

Procedure

1. Count cells using Burk chamber.
2. Plate 30,000 cells in each plate (six inches diameter).

3. Add to culture medium [U-¹⁴C] lactate (2 µCi/ml, final concentration).
4. Place the cells in incubator at 37 °C, 5% CO₂ for 24 h.
5. Wash cells with 2 ml of PBS.
6. Add to cells 1 ml of 20% trichloroacetic acid.
7. Rupture the cells with the scrapers.
8. Recover the cells and put them in an eppendorf tube.
9. Leave in ice for 30 min.
10. Centrifuge at 12,000 x g for 15 min at room temperature.
11. Remove the supernatant and resuspend the pellet with 200 µl of water.
12. Put 2 ml of scintillation liquid in a vial for scintillation.
13. Transfer an equal volume of resuspended pellet in the vial containing the scintillation liquid.
14. The resuspended pellet was assayed for [¹⁴C] labelled proteins by liquid scintillation counting.

Acknowledgments

This protocol is adapted from Fiaschi *et al.* (2012).

References

1. Fiaschi, T., Marini, A., Giannoni, E., Taddei, M. L., Gandellini, P., De Donatis, A., Lanciotti, M., Serni, S., Cirri, P. and Chiarugi, P. (2012). [Reciprocal metabolic reprogramming through lactate shuttle coordinately influences tumor-stroma interplay](#). *Cancer Res* 72(19): 5130-5140.