Detection of Released CO_2 by Radioactive Lactate

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[Abstract] This method allows to evaluate the degradation of lactate during cellular respiration. During this metabolic process, carbon atoms of lactate can be transformed to carbon dioxide. For this purpose, the radioactive lactate is added to the cells and the amount of radioactive carbon dioxide liberated is monitored. The radioactive carbon dioxide generated during cellular respiration is released into the culture medium and it is further converted into gas through the addition of sulfuric acid to culture media. A piece of Whatman paper wet with phenyl-ethylamine-methanol is placed inside the petri dish to trap radioactive carbon dioxide whose production is then evaluated by scintillator counting.

Materials and Reagents

1. Petri dish for cell culture (six inches diameter plate)
2. [U-^{14}C] lactate (U: uniformly labelled) (PerkinElmer, catalog number: NEC599250UC)
3. 2-Phenethylamine (Sigma-Aldrich, catalog number: P2641)
4. Methanol
5. 4 M H_2SO_4
6. Liquid scintillation (PerkinElmer, catalog number: 6NE9529)
7. Vials for scintillator (PerkinElmer)
8. Dulbecco's Modified Eagle Medium (DMEM)
9. Phenyl-ethylamine-methanol (1:1:1)

Equipment

1. Incubator for cell culture
2. Scintillator
3. Burker chamber
4. 3 mm Whatman paper

Procedure

1. Count cells using Burker chamber.
2. Plate the same number of cells in each plate (30,000 cells).
3. Wet a disk of Whatman paper with 100 µl of phenyl-ethylamine-methanol (1:1:1).
4. Put the wetted piece of Whatman paper under the lid of Petri dish.
5. Add to 1 ml of culture medium 0.2 µCi/ml D-[U-^{14}C] lactate.
6. Place the cells in incubator at 37 °C, 5% CO_2 for 15 min.
7. Add 200 µl of 4 M H_2SO_4 to culture medium.
8. Place 2 ml of scintillation liquid in a vial for scintillation.
9. Remove the Whatman paper and put it in the scintillation liquid.
10. Radioactive CO_2 released was counted by scintillator.

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.

