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Radiolabeled spermine uptake in cells

Forked from Radiolabeled polyamine uptake in cells

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We use this protocol and it's working

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Abstract

This protocol provides a technique to determine radiolabeled spermine uptake in cells, via the acquisition of counts per minute (CPM) using a Liquid Scintillation Counter.

Guidelines

Proper guidelines for working with radiolabeled materials should be followed at all times.

Materials

- ^{14}C-labeled spermine: 3139-50 µCi, ARC
- Cold spermine: 85590-5G, Merck
- RIPA Lysis and Extraction Buffer: 89900, Invitrogen
- EcoLite Liquid Scintillation Cocktail: 01882475-CF, MP Biomedicals

Safety warnings

Radiation hazards

1 Cells are seeded in 12-well plates, such that 70-80% confluency is reached on the day of the assay. Seed out 2 'treatment' wells and 1 'background' well per cell line.

2 Remove the culture medium from all wells.

3 In the treatment wells: add 300 µL of medium, containing 5 µM radiolabeled spermine.
In the background wells: add 300 µL of medium, containing 5 µM radiolabeled spermine and 100 µM unlabeled (cold) spermine.

4 Incubate 37 °C 00:30:00 30m

5 Aspirate the medium.

6 Wash wells 2x with 1 mL PBS (-/-).

7 Remove the last wash, then add 200 µl RIPA buffer to the wells.

8 Incubate 00:10:00 at Room temperature . 10m

9 Scrape the cells and pipette the lysates into scintillation vials, that are filled with 7 mL of scintillation fluid (Ecolite (+), MP). Rinse each well with 200 µL PBS (-/-) and pipette into the respective scintillation vial.

10 Mix scintillation vials well prior acquisition of counts per minute (CPM) in the Liquid Scintillation Counter.