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Radiolabeled polyamine uptake in cells

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ABSTRACT

This protocol provides a technique to determine the radiolabeled polyamine uptake capacity in cells, via the acquisition of disintegrations per minute (DPM) using a Liquid Scintillation Counter.

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GUIDELINES

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

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, 1 'quick wash' well and 1 'untreated' well per cell line and treatment dose.

A	B	C	D
treatment	treatment	quick wash	untreated

2 Remove the culturing medium in 'treatment wells' and add **500 µL** of medium, containing the desired concentration of radiolabeled polyamines. Leave regular culturing medium in the 'quick wash' and 'untreated' wells.

3 Incubate **37 °C** **00:30:00** 30m

4 4 minutes before reaching the 30-minute mark, perform a Quick-wash step as follows: add **500 µL** of medium, containing the desired concentration of radiolabeled polyamines in the 'quick wash' wells. **00:00:00** immediately remove the treatment medium, and wash with **500 µL** of DPBS (-/-) containing **50 micromolar (µM)** of the respective unlabeled (=cold) polyamine at **4 °C** . Continue with 2 more washing steps with **800 µL** of DPBS (-/-) **4 °C** .

5 Proceed with washing the other wells ('treatment' and 'untreated'). Remove the treatment medium, and wash with cold **500 µL** of DPBS (-/-) containing **50 micromolar (µM)** of the respective cold polyamine at **4 °C** . Continue with 2 more washing steps with **800 µL** of DPBS (-/-) at **4 °C** .

6 After the last wash, add **200 µL** 0.1% SDS-DPBS (-/-) per well to lyse the cells.

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10m

7 Incubate **00:10:00** at **Room temperature** .

8 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** DPBS (-/-) and pipette into the respective scintillation vial.

9 Mix scintillation vials well prior acquisition of disintegrations per minute (DPM) in the Liquid Scintillation Counter.

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