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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 the radiolabeled polyamine uptake capacity in cells, via the acquisition of disintegrations per minute (DPM) using a Liquid Scintillation Counter.

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.

Note

	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 [M] 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 [M] 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.

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

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.