LIPID MAPS DNA Assay

LIPID MAPS Protocol ID PP0000002700
05-23-2006

Supplies

- Quant-iT DNA Assay Kit, Broad Range (Molecular Probes, cat #Q-33130)
- CyQUANT Cell Lysis Buffer (Molecular Probes, cat #C-7027)

Harvest and Storage

1. Grow and treat cells as outlined in LIPID MAPS Protocol PP0000001003.
2. For 6 cm dishes, scrape cells into 2 or 3 mL PBS.*
3. Transfer cells in PBS to an appropriate vessel. Pipette 20X to suspend cells.
4. Remove an aliquot of cells to an Eppendorf tube for DNA assay.
5. To the DNA aliquot, add 5% volume of 50% ethanol.**
6. Vortex and freeze. Samples can be stored indefinitely.

DNA Assay

1. Thaw cells for assay. Vortex vigorously to resuspend. Visually confirm the suspension of cells in buffer.
2. Prepare the assay buffer:
    - Prepare 1X solution of CyQUANT Cell Lysis Buffer using 20X buffer solution and Millipore H_2O.
    - Dilute Quanti-iT DNA BR reagent 1:200 into 1X Cell Lysis Buffer.
3. Load 200 µL of assay buffer into each microplate well.
4. Add 5 µL of standard to standard wells.
5. Add 10 to 20 µL of cells to each well. Before pipetting each sample, vigorously pipette 5X with a P200 to ensure cell suspension. Use a volume of cells that will bring your assay values to the center of the standard curve.
6. Mix the plate well.
7. Measure the fluorescence using a microplate reader as outlined in the Quant-iT DNA Assay Kit brochure.

* Using 1 mM EDTA in PBS appears to have minimal effect on assay results, but may have unknown effects on cell metabolism.

* Recommended by Alex Andreyev.

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Date: 05-23-2006