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# Cell Viability Assay (MTT Assay) Protocol

aasirvatham[1]

[1]Department of Biology, Misericordia University

aasirvatham

ABSTRACT

The purpose of this experiment is to investigate the effects of forskolin-mediated cAMP activation on the viability of LPS-treated Schwann cells. The immortalized rat RT4-D6P2T (ATCC #CRL-2768) and S16 (ATCC #CRL-2941) cell lines were cultured and received one of the following treatments: 0.1, 1, or 10 µg/mL of LPS, in N2 media (control) or N2 media supplemented with 2 µM of forskolin, for 1, 3, 12, or 24 hours, and the CyQUANT MTT Cell Viability Assay Kit (Thermo Fisher) was used to perform the viability assay.

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**Protocol status:** Working
We use this protocol and it's working

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## To perform one (1) MTT assay:

1 Aseptically culture immortalized rat RT4-D6P2T Schwann cells (ATCC, Cat #CRL-2768, Manassas, VA) or S16 Schwann cells (ATCC, Cat #CRL-2941, Manassas, VA) in Dulbecco's Modified Eagle Medium (DMEM) (ATCC, Cat #30-2002, Manassas, VA) supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher, Cat #16000044, Waltham, VA) and 1% penicillin/streptomycin (Pen-strep) (GIBCO, Cat #15140-015, Gaithersburg, MD)/amphotericin B (R&D Systems, Cat #B23192, Minneapolis, MN) at 37°C and 5% $CO_2$ in poly-L-lysine (PLL)-coated dishes.

2 At 80% confluency, split and seed cells into DMEM (200 µL DMEM/well) in a PLL-coated 96-well plate at a density of ~35,000 cells/well.

3 Incubate cells in DMEM for 24 hours.

4 After 24 hours, aspirate the DMEM and wash each well 2-3x with 200 µL HBSS. After the last wash, add 200 µL $N_2$ media (DMEM/F12, no phenol red [Thermo Fisher, Cat #21041025, Waltham, MA] supplemented with 5 µg/mL insulin [Sigma, Cat #91077C, St. Louis, MO] and 100 µg/mL apo-transferrin [Sigma, Cat #T1147, St. Louis, MO]) to each well.

5 Incubate cells in $N_2$ media for 24 hours.

6 After 24 hours, prepare the forskolin-supplemented media by adding 5 µL of a 2 mM forskolin stock to 10 mL of $N_2$ media.

7 Add 200 µL of the appropriate medium to each well following the plate layout.

8 After adding the media, add the appropriate LPS dose to each well following the plate layout. For a 0.1 µg/mL dose of LPS, add 2 µL of a 10 µg/mL LPS stock OR 20 µL of a 1 µg/mL LPS stock. For a 1 µg/mL dose of LPS, add 2 µL of a 100 µg/mL LPS stock OR 20 µL of a 10 µg/mL LPS stock. For a 10 µg/mL dose of LPS, add 2 µL of a 1 mg/mL LPS stock OR 20 µL of a 100 µg/mL LPS stock.

9 Allow cells to incubate in the different treatment combinations for the required incubation time (1, 3, 12, or 24 hours).

10 Prior to the end of the incubation period, prepare a 12-mM MTT stock solution by adding 1 mL of sterile PBS directly to the tube. Vortex to mix until the MTT powder has completely dissolved.

11 Wrap the vial of MTT solution in aluminum foil and store at 4°C until ready for use (good for up to 4 weeks).

12 After the incubation period, remove the 96-well plate from the incubator and warm the vial of MTT solution by rubbing in between hands for about 10 seconds.

13 Add 10 µL of MTT to each well, and incubate the plate for 2-3 hours.

14 At the hour-and-a-half mark, remove one bottle of SDS from the MTT assay kit and bring to room temperature by letting it sit for 20-30 minutes.

15 Once at room temperature, add 10 mL of a 0.01 M HCl solution to the bottle of SDS and slowly pipette the mixture to minimize bubble formation.

16 After the 2-hour incubation with MTT, remove the 96-well plate from the incubator and add 100 µL of the SDS/HCl mixture to each well.

17 Incubate the plate for an additional hour but continuously check for lysed cells with purple precipitate (could take up to 1.5-2 hours).

18 Once the cells have lysed completely, remove the plate from the incubator and read in the SpectraMax ABS Microplate Reader (Molecular Devices, San Jose, CA) at 570 nm.

19 Optical density is measured as an indicator of cell viability, with a higher optical density indicating more viable cells, and a lower optical density indicating less viable cells.