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Cell Viability Assay (MTT Assay) Protocol

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We use this protocol and it's working

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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 $\mu\text{g}/\text{mL}$ 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.

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% CO₂ 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 N₂ 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 N₂ 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 N₂ 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.