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We use this protocol and it's working

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CellTitre Glo 2.0 Viability Assay

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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, 0.1, 1, or 10 µg/mL of LPS, in N2 media (control) or N2 media supplemented with 2 µM forskolin for 1, 3, 12, or 24 hours. The CellTitre-Glo 2.0 Viability Assay (Promega) was used to perform the viability assay.

ATTACHMENTS

Plate Layout.jpg

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To perform one CellTitre-Glo Viability 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 ~20,000 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 200µL N2 media (DMEM/F12, no phenol red [Thermo Fisher, Cat #21041025, Waltham, MA] supplemented with5 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 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.

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

9 Prior to the end of the incubation period, remove the CellTitre-Glo 2.0 Reagent and allow to equilibrate to room temperature.

10 Allow cells to equilibrate to room temperature for approximately 30 minutes.

11 Add an equivalent volume of CellTitre-Glo 2.0 Reagent to the volume of cell culture medium in each well. Be sure this step is done in minimal light.

12 Mix the contents for 2 minutes to induce cell lysis.

13 Once the contents have been mixed, read the plate in the SpectraMax M4 Multi-Mode Microplate Reader (Molecular Devices, San Jose, CA)

14 Luminescence is measured as an indicator of cell viability, with a higher luminescence indicating more viable cells, and a lower luminescence indicating less viable cells.