Giardia Electrophysiological Assays

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Abstract

Methods for TransEpithelial Electrical Resistance (TEER) and short circuit current measurement of the effects of soluble mediator on intestinal epithelial cells.

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Guidelines

Before start

Prepare Forskolin (10µM), UTP (100µM), Amiloride (10µM), DIDS (100µM) and GlyH-101 (50 µM) in aqueous solution and Amiloride (10mM), GlyH-101 (50mM) in DMSO.

Protocol

Culture of the Human Colonic Adenocarcinoma Derived Epithelial Cell Line, CaCo-2

Step 1.

Grow CaCo-2 cells in 75 cm^2 flasks and maintain in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 20 % (vol/vol) heat inactivated foetal calf serum (FCS), nonessental amino acids, 1 % penicillin/streptomycin (12IU/ml penicillin/ 12µg/ml streptomycin) and gentamycin (47µg/ml). Culture cells at 37°C in a humidified atmosphere of 95% O_2 and 5 % CO_2. Change the medium every 48 to 72 hours to remove debris from flasks.

Culture of the Human Colonic Adenocarcinoma Derived Epithelial Cell Line, CaCo-2

Step 2.

Once 80% confluency is reached passage the cells by incubation with 0.25% trypsin/EDTA.

Seeding on Transwell Filters

Step 3.

Seed the CaCo-2 cells at a density of 6 x 10^4 cells/cm² in 6-well Transwell filters (0.4 µm pore size) and culture for 7-15 days until confluent. Then, use confluent monolayers for electrophysiological experiments, for co-culture experiments with Giardia parasites or for culture with Giardia supernatants.

TEER Measurement

Step 4.

Grow monolayers of CaCo-2 cells on 6-well Transwell filters (0.4 µm pore size) for 7-15 days until confluent. Assess the development of the polarised monolayer by measuring the TEER over a 7-15 day period. Measure TEER daily using the Millicell electrical resistance meter. Once confluent, add Giardia to the apical side of the Transwell filter and incubate for 24 hours. Assess the integrity of the confluent polarised monolayer by measuring the TEER before and/or after apical infection by Giardia.

Ussing Chamber Assays

Step 5.

Mount monolayers of CaCo-2 cells on Transwell filters into a Physiological Instruments EM-CSYS-2 Ussing chamber set-up, after establishment of a confluent monolayer and the short circuit current (ISC) across the monolayer is continuously measured.

Ussing Chamber Assays

Step 6.

Bathe both sides of the epithelium in 5ml of Krebs Henseleit solution that is continuously circulated through the half chambers, maintained at 37 °C and continuously bubbled with 95% O_2 / 5% CO_2.

NOTES

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The Krebs Henseleit bath solution has the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 11.1 (pH 7.4).

Ussing Chamber Assays

Step 7.

Leave the permeable supports for 30 mins to equilibrate before experiments are started.

Ussing Chamber Assays

Step 8.

Treat all filters with 10µM amiloride apically to eliminate electrogenic sodium absorption through epithelial sodium channels (ENaC). Monitor Isc continuously across the monolayers by a Physiological

Instruments Multichannel Voltage/Current Clamp (VCC MC6) through 3M KCl/agar, Ag/AgCl2 cartridge electrodes (Physiological Instruments), and record the raw data for Isc, transepithelial resistance and transepithelial voltage using Acquire and Analyse version 1.3 software (Physiological Instruments). Export data to Microsoft Excel initially and then into GraphPad Prism version 5.0 for Windows package for data representation and statistical analysis.

Warnings

These chemicals are toxic on contact or ingestion and should be handled and disposed of accordingly to MSDS sheet and Health and Safety regulation.