

MDCK Transwell Filter Monolayers

MDCK Media (makes 570ml; sterile filter prior to use; store in the dark at 4C):

<u>Component</u>	<u>Starting Concentration</u>	<u>Final Concentration</u>	<u>Volume</u>
DMEM	--	--	488ml
FBS	100%	10%	57ml
L-Glutamine	200mM	2mM	5.7 ml
Penicillin	100U/ml	0.5 U/ml	2.85ml
Streptomycin	100ug/ml	0.5ug/ml	2.85ml
Hepes buffer	1M	20mM	12ml

Filter Pre-Incubation

- 1) Pre-incubate Transwell filters at 37C with appropriate volume of MDCK media for at least 30 minutes prior to seeding (when adding media to basolateral compartment, do not remove Transwell insert from the well, but rather Pipette tip through one of the three openings on the side of the insert to add media)
 - a) 6.5mm filters (24-well plate, Fisher catalog number 07200154): add 100ul to apical compartments and 600ul to basolateral compartments
 - b) 24mm filters (6-well plate, Fisher catalog number 07200170): add 1.5ml to apical compartments and 2.6ml to basolateral compartments

Cell Seeding

- 1) Rinse MDCK (available from ATCC) in 10cm dish briefly with PBS.
- 2) Add 3ml trypsin/EDTA to dish and incubate at 37C.
- 3) Lift cells by tapping and check adherence under microscope.
- 4) Once >90% of cells have detached, add 3ml MDCK media to inhibit trypsin.
- 5) Transfer media/cells to 15ml conical tube
- 6) Centrifuge 500g x 5min
- 7) Remove supernatant and resuspend pellet in 1ml MDCK media
- 8) Count 15ul sample of cell suspension in hemacytometer
- 9) Seed filters with appropriate volume of cell suspension (make sure to leave some filters unseeded as controls for transport assay)
 - a) 1×10^5 cells per 6.5mm filter
 - b) 1×10^6 cells per 24mm filter
- 10) Change media daily for 4-6 days
 - a) Aspirate basolateral media first using Pasteur pipette
 - b) Aspirate apical media next using P200 tip over Pasteur pipette with minimal suction

Trans-Epithelial Electrical Resistance (TEER)

- 1) On experiment day, check TEER using EVOM resistance meter (World Percision Instruments)
 - a) Note: EVOM STX-2 electrode tips which come with the machine are only compatible with 6.5mm filters (24-well plate size).
- 2) Wipe down EVOM box, electrode tip and wire, and insert into tissue hood
- 3) Place electrode tips into 50ml conical filled with 5ml of 70% EtOH for 5 minutes
- 4) Set EVOM to Power = on; Range = 2000ohms; Mode = R.
- 5) Rinse EtOH off of electrode tip thoroughly with 1ml DMEM, PBS or MDCK media immediately prior to measuring resistance
 - a) Make sure to do this when switching between cell types, or when going from seeded to non-seeded filters.
- 6) Insert long electrode tip into basolateral compartment through one of the openings on the side of the filter and allow the shorter tip to sit in the apical compartment.

- 7) Press “Measure R” button and record resistance
- 8) Either discard or continue growing filters that have resistance < 200 ohms (non-transfected MDCK cells achieve TEER > 300 ohms, and transfected cells achieve TEER > 200 ohms)

A β Transport Assay (after 4-6 days of growth)

- 1) Dissolve 25uCi of [¹²⁵I]A β (GE Healthcare item IMQ.2049v) in 200ul MilliQ H₂O and freeze at –80C in 25ul aliquots of 62.5nm
- 2) Dissolve 50uCi of [¹⁴C]Inulin (GE Healthcare) in 2ml PBS and freeze at –80C in 45 & 90ul aliquots of 25uCi/ml.
- 3) On day of experiment, add 6-12ul of A β solution (625 – 1250pmol) or 6-12ul of inulin solution to basolateral side and gently shake for a few seconds to properly mix
 - a) If you are worried about inter-filter variability based on your TEER measurements, you can add inulin and A β to the same well.
- 4) At 30 minutes remove 10ul samples of apical and basolateral media of each well and put in scintillation vials for gamma (A β) or beta (inulin) counter.
- 5) Add 4.5ml EcoScint scintillation fluid to each beta counter vial
 - a) You do not need to add fluid to gamma samples, but make sure they are close to the bottom of the vial
- 6) Put on cap and vortex to suspend sample (only beta samples)
- 7) Gently wipe the vials with a KimWipe sprayed with ethanol to remove static electricity
- 8) Count vials on beta and/or gamma counter