Invasion Assay Protocol

Materials

- Transwell inserts (24-well plate, 8 µm pore size)
- Matrigel or basement membrane matrix
- Cell culture medium
- Crystal violet stain
- PBS (Phosphate Buffered Saline)
- Trypsin-EDTA
- SnapCyte™ software for quantification

Procedure

Transwell Insert preparation:

1. Coat the upper surface of Transwell inserts with 50 µL of Matrigel (diluted 1:3 in serum-free medium). If studying migration, skip the coating step.
2. Incubate the inserts at 37°C for 1 hour to allow the Matrigel to solidify.

Thaw Matrigel on ice and evenly coat the well for best results

Cell Seeding:

1. Harvest cells using trypsin-EDTA and resuspend in serum-free medium.
2. Seed 2.5 - 5 × 10^4 cells in 100 µL of serum-free medium onto the Matrigel-coated inserts.
3. Add 600 µL of medium containing 10% FBS (as a chemoattractant) to the lower chamber of the well.

Thoroughly wash off stain! Excess stain can highly interfere with accurate quantification

Incubation:

1. Incubate the plates at 37°C in a 5% CO2 incubator for 24-48 hours, depending on cell type and expected invasiveness.

Optimize inucbation time: **Highly invasive cells** may require shorter incubation periods, while **less invasive cells** may need extended periods to migrate through the matrix

Fixation and Staining:

1. Remove the non-invaded cells from the upper surface of the insert using a cotton swab.
2. Wash the inserts gently with PBS.
3. Fix the invaded cells on the lower surface of the insert by immersing in 70% ethanol for 10 minutes.
4. Stain the cells with 0.1% crystal violet for 10 minutes at room temperature.
5. Wash the inserts thoroughly with distilled water to remove excess stain.
6. Allow the inserts to air dry.

Include both positive and negative controls in your assay!
Positive Controls = cells known to invade efficiently
Negative Controls = cells with inhibited invasion capability

Quantification

1. Set up your experiment groups and replicates on the SnapCyte™ app or web app and define your control group (optional).
2. Acquire 4 images (at 10x) per well using a standard microscope with SnapCyte™ adapter and SnapCyte™ app, or any digital microscope available in your lab, and upload to SnapCyte™ web app.
3. Adjust or set ROIs on images if necessary.
4. Data: Invasion cell density or normalized ratio to your set control can be exported as an Excel file or graph.

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