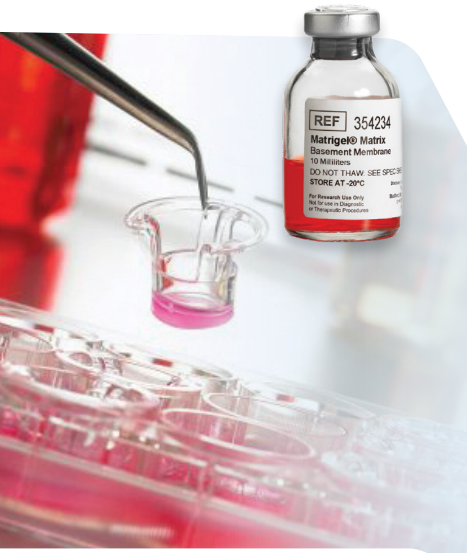
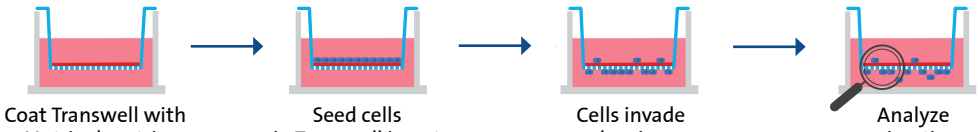
Mastering Corning® Transwell® Migration Assays

Setting Up Corning Transwell Permeable Supports for Migration or Invasion Assays

1. Transwell inserts come pre-assembled in a companion plate. For invasion assays, use a sterile syringe or pipet to apply extracellular matrix to inserts, following manufacturer's instructions to form a barrier.
2. Seed cells or spheroids into the upper chamber at the desired density and volume.
3. Place desired attractant in the lower chamber in the appropriate media.
4. Incubate for the desired time for migration and/or invasion to occur.
5. Collect or fix, stain, and image cells to quantify migration or invasion.





Materials Checklist for Migration or Invasion Assays using Transwell Permeable Supports

- Transwell inserts (comes pre-assembled in a plate)
- Extracellular matrix, such as Corning Matrigel® matrix (for invasion assays only)*
- Cells or spheroids whose migration or invasion will be tested
- Non-invasive or non-migratory control cells
- Attractant (such as a cancer spheroid or FBS in media)
- Sterile forceps
- Pipettor and pipet tips
- Culture media
- Incubator
- Cell harvesting and staining supplies
- Analysis device and supplies (such as microscope, plate reader, or flow cytometer)

*See protocol (CLS-DL-CC-031) for optimal coating concentration of Corning Matrigel matrix.

Choosing an Analysis Method

Adherent cells?

NO Remove inserts and collect cells from the underside of the membrane. Perform quantification by counting or staining and imaging cells using hemacytometer, flow cytometer, or plate reader.

YES *High throughput needed?*

NO Use a cotton swab to remove non-invaded or non-migrated cells on the upper surface of the insert. Then, fix and stain cells that have migrated to the underside of the membrane. Quantify using microscopy.

YES Dissociate migrated cells from the underside of the membrane, and use calcein AM and a plate reader to quantify cells.

OR Use **crystal violet to stain cells** on the membrane; then, use acetic acid to elute the dye. Measure absorbance using a plate reader.

Troubleshooting Common Challenges

- If cells are not migrating adequately, ensure cells or their receptors haven't been damaged by harvesting procedures. Or, try serum starving cells for 24 to 48 hours to increase sensitivity to the chemoattractant.
- If too few or too many cells migrate, ensure the pore size is correct.
- Too low seeding density can lead to low measurable response; seeding density can cause oversaturation of pores or assay signal. Adjust cell seeding density accordingly.

Expert Tips for Successful Transwell Assays

- Include appropriate controls.
- Optimize experiments by titrating cell density, chemoattractant concentration, and migration time.
- Consider testing different chemoattractants, invasion barriers, or invasion barrier concentrations.
- If quantifying via microscopy, include enough fields near the center and edges of the insert.

For more information, see Considerations when Optimizing your Chemotaxis or Invasion Assay with Corning Transwell Permeable Supports.

www.corning.com/lifesciences/transwells

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