



Supporting Protocol

Migration Assay with TissueSpec® ECM Hydrogel

Migration is essential for the dissemination of tumor cells from primary solid tumors to secondary sites in distant organs. Mechanisms regulating the directed movement of cells in metastasis can be studied using a migration assay. This protocol may be used to prepare TissueSpec® ECM Hydrogels, cells, and migration chambers (**Figure 1**) for analysis of migration.

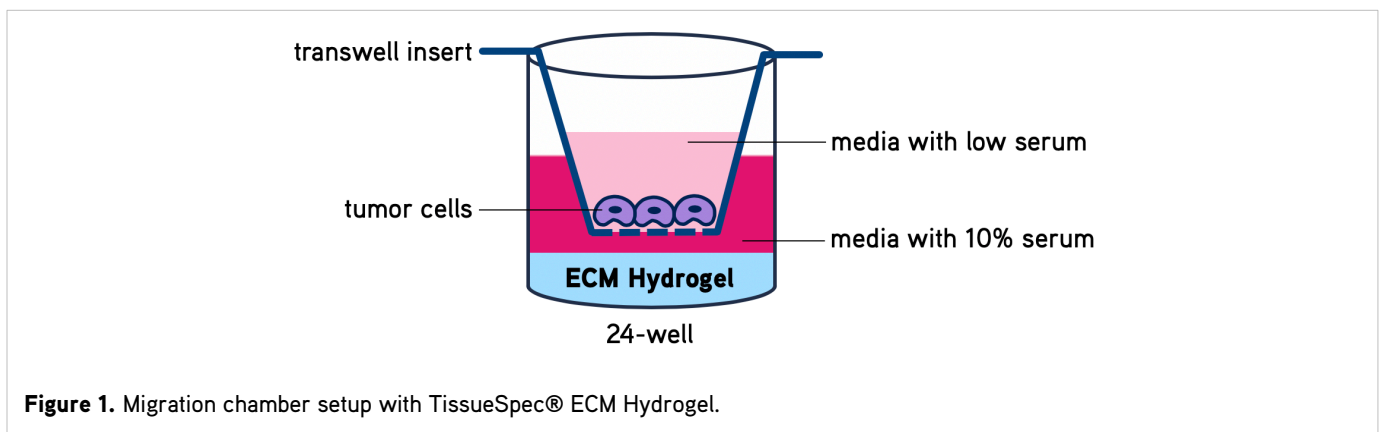


Figure 1. Migration chamber setup with TissueSpec® ECM Hydrogel.

Procedure

Preparation of TissueSpec® ECM Hydrogel

1. Add 30 μL Component A into one of the ECM Component tubes containing 300 μL ECM.
2. Mix thoroughly by vortexing.
3. Add 35 μL Component B into the ECM Component tube containing ECM and component A.
4. Mix thoroughly by vortexing.
5. Add 135 μL cell culture media into the ECM Component tube containing components A and B.

Note: While we recommend preparation of ECM Hydrogel at 6 mg/mL, final hydrogel concentration can be adjusted by varying the volume of cell culture media.

6. Mix thoroughly by vortexing.
7. In a 24-well plate, apply a thin layer of TissueSpec® ECM Hydrogel (~200 μL per well) to cover the surface of each well.
8. Incubate the 24-well plate at 37°C in a humidified environment with 5% CO₂ for 30 minutes to achieve gelation.

Preparation of Cells

1. Trypsinize cells.
2. **Optional:** Label cells with fluorescent label (e.g., 1 μ L CellTracker in 1 mL serum-free media).
3. Prepare cell suspension at desired concentration using low-serum media (e.g., 0.5% fetal bovine serum).

Preparation of Migration Chamber

1. After ECM Hydrogel achieves gelation, place a transwell insert into each hydrogel-coated well of the 24-well plate.

Note: Pore size of transwell inserts may vary according to experimental design and cell type (e.g., pore size of 5 – 8 μ m for tumor cells or fibroblasts).

2. Add 300 μ L cell suspension into each transwell insert.
3. Add 800 μ L media supplemented with 10% serum into the bottom of each well of the 24-well plate.
4. Incubate the 24-well plate at 37°C in a humidified environment with 5% CO₂ for 24 hours.

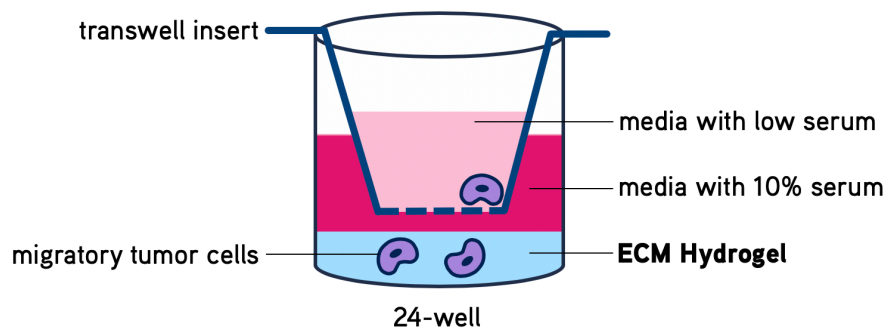


Figure 2. Migration of tumor cells into TissueSpec® ECM Hydrogel.

Analysis of Migration

1. After 24 hours, remove transwell insert from each well.
2. Capture images of cells that migrated to the ECM hydrogel.
3. Quantify the number of cells that migrated to the ECM hydrogel.