Supporting Protocol

Invasion Assay with TissueSpec® ECM Hydrogel

Invasion of cancer cells into neighboring tissues is a critical process in tumor cell dissemination and formation of metastases. Mechanisms regulating cell invasion in metastasis can be studied using an invasion assay. This protocol may be used to prepare TissueSpec® ECM Hydrogels, cells, and invasion chambers (**Figure 1**) for analysis of invasion.

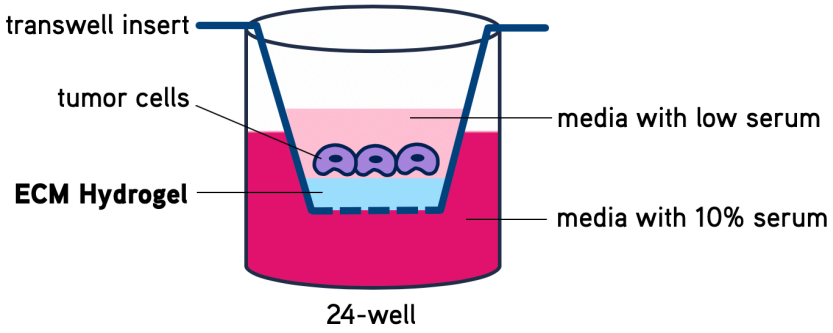

Figure 1. Invasion chamber setup with TissueSpec® ECM Hydrogel.

Procedure

Preparation of TissueSpec® ECM Hydrogel

1. Place a transwell insert into each 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 µm for tumor cells or fibroblasts)

2. Add 30 µL Component A into one of the ECM Component tubes containing 300 µL ECM.

3. Mix thoroughly by vortexing.

4. Add 35 µL Component B into the ECM Component tube containing ECM and component A.

5. Mix thoroughly by vortexing.

6. 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.

7. Mix thoroughly by vortexing.

8. Add 80 µL TissueSpec® ECM Hydrogel into each transwell insert.

9. Incubate the 24-well plate with transwells at 37˚C in a humidified environment with 5% CO_2 for 45 minutes to achieve gelation.

Preparation of Cells

1. Trypsinize cells.
2. **Optional**: Label cells with fluorescent label (e.g., 1 µL 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 Invasion Chamber

1. Add 800 µL media supplemented with 10% serum into the bottom of each well of the 24-well plate.
2. After ECM Hydrogel achieves gelation, add 300 µL cell suspension at desired concentration on top of each hydrogel.
3. Incubate the 24-well plate at 37˚C in a humidified environment with 5% CO_2 for 24 hours.

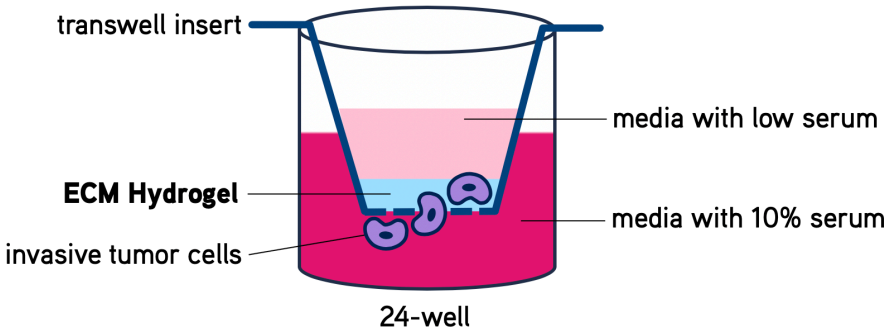

Figure 2. Invasion of tumor cells through TissueSpec® ECM Hydrogel.

Analysis of Invasion

1. After 24 hours, capture images of cells that invaded the ECM hydrogel.

   Note: If cells were labeled with a fluorescent label (e.g., CellTracker), use fluorescence microscopy to visualize invasive cells. If cells were not labeled with a fluorescent label, remove transwell inserts, carefully remove hydrogels using a cotton swab, then fix and stain with Crystal violet the cells remaining on the underside of the transwell insert.

2. Quantify the number of cells that invaded the ECM hydrogel.