SARTORIUS

Protocol

Detailed Incucyte® Chemotaxis Cell Invasion Assay Protocol

The following protocol is a detailed example designed to enable you to run a successful transmembrane cell invasion assay using the Incucyte® Chemotaxis Cell Invasion Assay. Note that the protocol does not include a description of any experiments required for optimization. Here we specifically describe the use of the Incucyte® Live-Cell Analysis System for establishing and quantifying chemotactic invasion of HT-1080 cells.

Materials

- Incucyte® Nuclight Red HT-1080 (Sartorius Cat. No. 4485) cells in culture (T75, 37 °C, 5% CO_2) in:
  - Growth medium: F-12 (Invitrogen 11765) + 10% FBS + 1% Glutamax + 1% Pen/Strep. + 0.5 µg/mL Puromycin
  - Assay medium: F-12 (Invitrogen 11765) + 1x Insulin-Transferrin-Selenium (ITS, Life Technologies 41400-045)
- Incucyte® Clearview 96-well Plate (Sartorius Cat. Nos. 4582 or 4599)
- 2X Incucyte® Clearview 96-well Reservoir Plate (Sartorius Cat. Nos. 4600 or 4601)
- 0.25% Trypsin/EDTA (Life Technologies 25200)
- D-PBS (w/o Ca^{2+}, Mg^{2+}, Life Technologies 10010)
- Fetal Bovine Serum (FBS, Sigma-Aldrich F2442-500 mL)
- Cultrex® 3D Culture Matrix™ Reduced Growth Factor Basement Membrane Extract (Trevigen 3445-005-01)
- Incucyte® Chemotaxis Cell Invasion Accessories Kit (Sartorius Cat. No. 4444)
  - 2X CoolBox® 96F System configured with CoolSink® 96F
  - CoolBox® 30 System configured with CoolRack® M30
  - CoolSink® 96F for 96-well flat bottom plates

Protocol

Prepare Plate

Notes: Prior to initiating the invasion assay, cool centrifuge to 4 °C (if possible) and place a CoolSink® microplate insert on the frozen cold pack within the CoolBox®.

1. Thaw Basement Membrane Extract (BME) on ice or in a pre-chilled CoolBox®. Once thawed, keep on ice until ready to use.

   Note: BME will gel at temperatures above 8 °C. It is extremely important to keep this reagent at 4 °C to prevent polymerization during assay set up.

2. Pre-cool an Incucyte® Clearview 96-well Plate in a CoolBox® system containing a frozen cold pack and CoolSink® plate (4 °C), for 5 minutes.

   Note: It is important to keep close temperature control of the Incucyte® Clearview Plate. The Incucyte® Cell Invasion Accessories Kit (Cat. No. 4444) includes a specialized CoolBox® system to ensure the temperature of your assay plate and biomatrix materials are maintained between 4–8 °C—preventing premature polymerization and eliminating edge effects. Crushed ice can be used as an alternative however non-uniform cooling can lead to assay variability.

3. Add 150 µL of D-PBS (4 °C) to each well of the pre-chilled Incucyte® Clearview Reservoir Plate. Replace the Incucyte® Clearview insert and allow the membrane to prime for 20 minutes within the CoolBox®.
4. While the Incucyte® Clearview Plate is priming, dilute the BME to 5 mg/mL using chilled assay medium.

   Note: This reagent will be used at a volume of 20 µL per insert well, but prepare the volume you need with excess (e.g., prepare 4 mL biomatrix solution to provide 20 µL per insert well).

Prepare Cells

5. Remove medium from culture and gently rinse with D-PBS. Harvest cells and perform a cell count (e.g., trypan blue staining + hemocytometer).
6. Transfer 200,000 cells to a 15 mL conical tube and centrifuge at 1,000 rpm for 4 minutes.
7. Resuspend the cell pellet in 4 mL of BME (5 mg/mL) (prepared in Step 4). Final cell stock will be 50,000 cells/mL. Keep on ice at all times.

Set Up Plate

8. Using a multi-channel pipette and reverse pipetting technique, seed cells (20 µL per well, 1,000 cells per well) into every well of the Incucyte® Clearview insert in the CoolBox® (4 °C).

   Note: Use a pre-cooled reservoir boat when seeding cells.
9. Centrifuge Incucyte® Clearview Plate in a cooled centrifuge (set at 4 °C) for three minutes at 50 x g.
10. Place the Incucyte® Clearview Plate at 37 °C on a pre-warmed CoolSink® and allow the BME to polymerize for 30–60 minutes.
11. During incubation, prepare chemoattractants (e.g. 2-fold serial dilutions of 10% FBS in F-12 medium + ITS). Assay medium alone (F-12 + ITS) should be used as a negative control.
12. Using a manual multi-channel pipette, add 200 µL of the chemoattractants and control assay medium to the appropriate wells of the second reservoir plate.
13. Using a manual multi-channel pipette and reverse pipetting technique, gently add 40 µL of assay medium to all insert wells on top of the BME/cell layer.
14. Carefully transfer the insert into the pre-loaded reservoir plate. Be careful not to introduce bubbles which can become trapped below the membrane when placing the insert into the pre-filled reservoir plate.

Initiate Scanning

15. Place the Incucyte® Clearview Plate into the Incucyte® Live-Cell Analysis System and allow the plate to warm to 37 °C for at least 15 minutes. After 15 minutes, wipe away any condensation that remains on the outside of the plate lid or bottom of the reservoir.
16. In the Incucyte® software, schedule 24 hour repeat scanning (10x) every 2–3 hours. Typical assay duration is 72 hours.
    a. Objective: Ensure 10x objective is installed
    b. Vessel Type: Select "Incucyte® Clearview 96-Well Plate"
    c. Channel Selection: Select "Phase" + "Red" (800 ms acquisition time)
    d. Scan Mode: Select "Chemotaxis (Top/Bot)" scan type and desired Scan Pattern

       Note: The Incucyte® Live-Cell Analysis System estimates a scan time of 33 minutes per plate (phase and red); however, the actual scan time can take longer.

Note: We recommend plotting bottom side fluorescent nuclear count as the assay metric.

All	1	2	3	4	5	6	7	8	9	10	11	12
A	FBS 10% HT-1080 (1) 1K/well Migration Assay Buffer on Top		FBS 5% HT-1080 (1) 1K/well Migration Assay Buffer on Top		FBS 2.5% HT-1080 (1) 1K/well Migration Assay Buffer on Top		FBS 1.25% HT-1080 (1) 1K/well Migration Assay Buffer on Top		FBS 0.63% HT-1080 (1) 1K/well Migration Assay Buffer on Top		HT-1080 (1) 1K/well Migration Assay Buffer on Top	
B												
C												
D												
E	FBS 10% HT-1080 (1) 1K/well Migration Assay Buffer on Top		FBS 5% HT-1080 (1) 1K/well Migration Assay Buffer on Top		FBS 2.5% HT-1080 (1) 1K/well Migration Assay Buffer on Top		FBS 1.25% HT-1080 (1) 1K/well Migration Assay Buffer on Top		FBS 0.63% HT-1080 (1) 1K/well Migration Assay Buffer on Top		HT-1080 (1) 1K/well Migration Assay Buffer on Top	
F												
G												
H												

Total HT-1080 Area (µm²/Well) [Bottom]

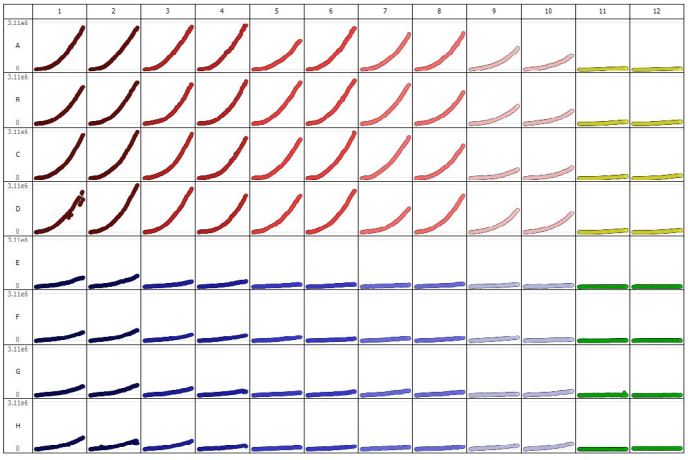

Figure 1: Determination of Incucyte® Nuclight Red HT-1080 Invasive and Migratory Response Toward FBS. Plate map and corresponding microplate graph for FBS agonist curves. Serum starved Incucyte® Nuclight Red HT-1080 cells were either embedded in 5 mg/mL BME (invasion) or seeded directly onto the Incucyte® Clearview Plate membrane (migration control) at 1,000 cells per insert well. The indicated concentration of FBS was added to the reservoir plate and data were collected for 96 hours at 2-hour intervals. Each well is individually graphed illustrating the difference in kinetic movement of migrating and invading cells.

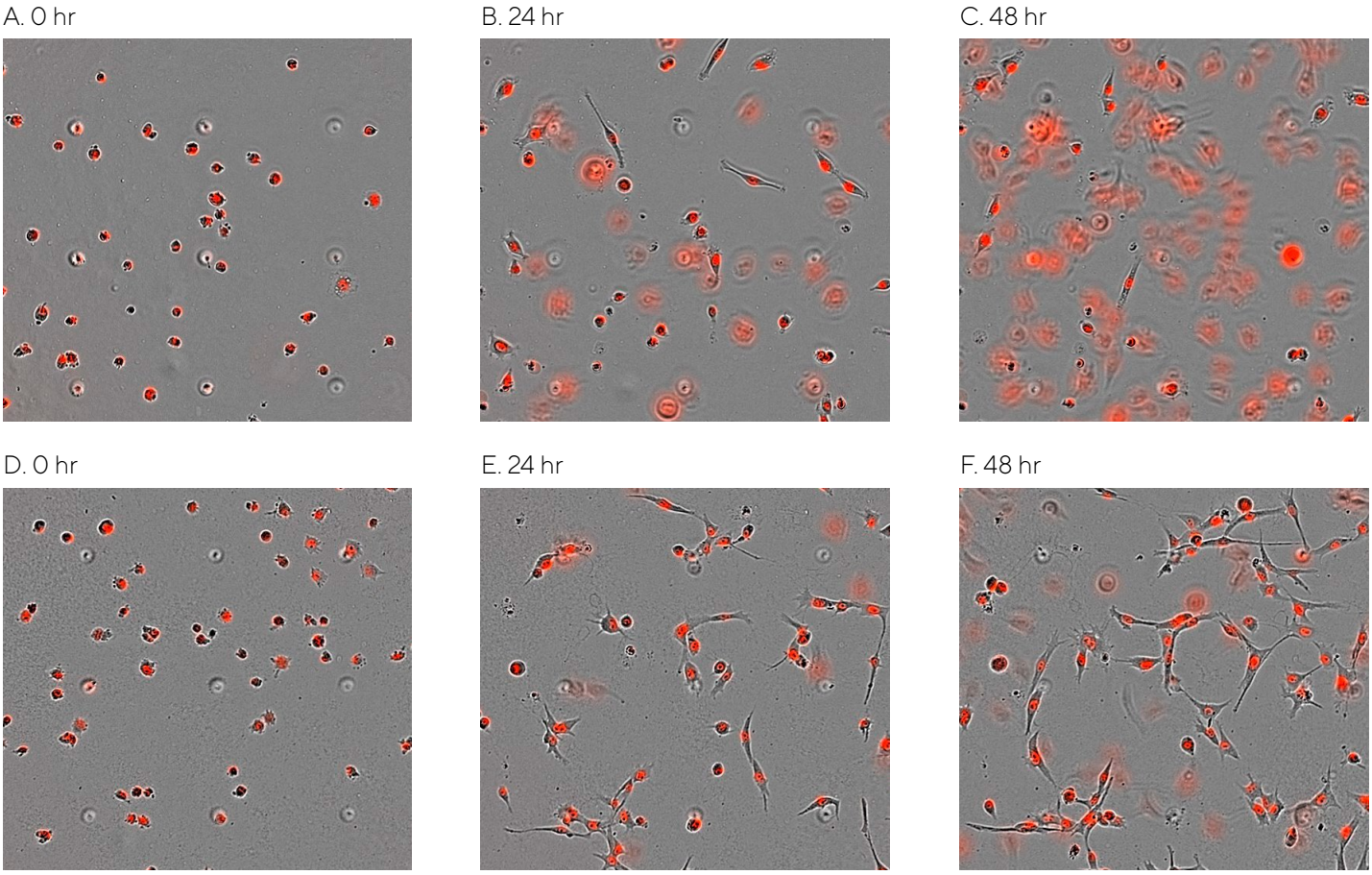

Figure 2: Representative Morphological Images of HT-1080 Cells. Migration: Images A, B, and C show the time-lapse progression of HT-1080s cells migrating toward 10% FBS. HT-1080 cells have a flat appearance and migration to the bottom side of the membrane (cells that are out of focus) can be seen prior to 24 hours. Invasion: Images D, E, and F show HT-1080 cells invading through the BME (5 mg/mL) toward the membrane pores. Cells have an elongated morphology, sending out laemellipodia or extensions to drive movement through a dense gel layer.

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