

Extravillous Trophoblast Migration and Invasion Assay

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[Abstract] Extravillous trophoblast (EVT) migration and invasion through the decidualized endometrium is essential to successful placentation. SGHPL-4 cells, an EVT cell line derived from first trimester placenta, is a widely used model of cytotrophoblast differentiation into an invasive phenotype. Here we describe a quantitative cell migration assay that can be modified to also measure cell invasion. SGHPL-4 cells were seeded into BD Fluoroblok cell culture inserts constructed with an 8 µm porous membrane and allowed to migrate towards epidermal growth factor, a known chemoattractant for EVTs. To assess EVT invasion, Fluoroblok inserts were first coated with Matrigel, a basement membrane matrix. SGHPL-4 cells were labeled with calcein AM and cells that had invaded and/or migrated across the membrane were quantified by a bottom-reading fluorescence plate reader. The advantage of the Fluoroblok inserts over other migration/invasion assays is that they allow nondestructive detection of migrated cells.

Materials and Reagents

1. SGHPL-4 cells (Kindly provided by Dr. Guy Whitley, St. George's University of London)
2. Ham's F10 Nutrient Mix (Life Technologies, Invitrogen™, catalog number: 11550-043)
3. Fetal bovine serum (FBS)
4. Dulbecco's Phosphate-Buffered Saline (DPBS) without Ca²⁺ and Mg²⁺ (Life Technologies, Invitrogen™, catalog number: 14190)
5. TrypLE Express (Life Technologies, Invitrogen™, catalog number: 12604013)
6. Matrigel, Growth Factor Reduced, Phenol Red Free (BD Biosciences, catalog number: 356231)
7. Recombinant Human Epidermal Growth Factor (hEGF) (BD Biosciences, catalog number: 354052)
8. BD Falcon HTS FluoroBlok Inserts (BD Biosciences, catalog number: 35112)
9. Calcein AM (Life Technologies, Invitrogen™, catalog number: C3100MP)

10. Hank's balanced salt solution (HBSS) (Life Technologies, Invitrogen™, catalog number: 14025)

Equipment

1. Centrifuges
2. 37 °C, 5% CO₂ Cell culture incubator
3. Inverted Fluorescent Microscope
4. Fluorescent plate reader

Procedure

DAY 1

1. For Invasion Assay, pre-Coat Fluoroblok Filter (8 µm porous membrane)
 - a. Prechill Fluoroblok inserts, companion plates and pipet tips to help maintain Matrigel in the liquid state.
 - b. Place desired number of prechilled inserts into a 24-well companion plate.
 - c. Add 50 µl of 1:10 Matrigel (diluted in HamF10) to each transwell insert.
 - d. Incubate at 37 °C, 3 h.
2. Serum starve cultures (70-75% confluent) for 24 h in 0.5% FBS/HamF10
 - a. Aspirate media.
 - b. Wash with 7 ml warm DPBS (without Ca²⁺ and Mg²⁺).
 - c. Add 12 ml warm 0.5% FBS/HamF10.
 - d. Incubate cells for 24 h at 37 °C.

DAY 2

1. Prepare cells (Upper Chamber)
 - a. Rinse cells once with 10 ml DPBS (without Ca²⁺ and Mg²⁺); add 3 ml TrypLE Express and incubate at 37 °C for 3-5 min; add 7 ml 0.5% FBS/HamF10 → 10 ml total.
 - b. Count cells using a hemacytometer.
 - c. In a 50 ml conical tube, centrifuge cells at 300 x g for 10 min.
 - d. Remove supernatant and resuspend cells in 0% FBS/HamF10 to obtain a cell suspension concentration of 1.2 x 10⁶ cells/ml (or 1,250 cells/µl).
 - e. Cap tube and store at room temperature till ready to load in chamber.
2. Prepare the chemoattractant (Treatments in Bottom Chamber)
 - a. Dilute desired chemoattractant in 0% FBS/HamF10. You will need 800 µl per well.
 - b. Prepare 10 ng/ml EGF as positive control.

- c. Add 800 μ l of chemoattractant to the bottom of each well. Avoid bubbles.
3. Assemble invasion chamber
 - a. Using a forceps, carefully remove insert from empty well.
 - b. Add 200 μ l of cells (2.5×10^5 for Invasion Assay or 5×10^4 for Migration Assay) to Matrigel-coated (for Invasion Assay) or uncoated insert (for Migration Assay).
 - c. Lower the insert at an angle into the well containing the chemotactic substance. Check for bubbles by looking under the plate. If there are bubbles, remove insert and try again.
 - d. Incubate at 37 °C for 12 h for Cell Migration Assay or 20-22 h for Cell Invasion Assay.

DAY 3

1. After invasion period, label invaded cells (on lower side of filter) with Calcein AM. For each well, add 2 μ l of Calcein AM to 500 μ l of HBSS.
2. Carefully aspirate the media from the insert, without disturbing the Matrigel layer.
3. Transfer the insert to a fresh well containing Calcein AM/HBSS solution.
4. Incubate at 37 °C for 1 h in the dark.
5. Read plate on fluorescent plate reader at 520 nm or take pictures using an epifluorescent microscope.

Acknowledgments

This protocol is adapted from Angelova *et al.* (2012).

References

1. Angelova, M., Zvezdaryk, K., Ferris, M., Shan, B., Morris, C. A. and Sullivan, D. E. (2012). [Human cytomegalovirus infection dysregulates the canonical Wnt/beta-catenin signaling pathway](#). *PLoS Pathog* 8(10): e1002959.
2. LaMarca, H. L., Ott, C. M., Honer Zu Bentrup, K., Leblanc, C. L., Pierson, D. L., Nelson, A. B., Scandurro, A. B., Whitley, G. S., Nickerson, C. A. and Morris, C. A. (2005). [Three-dimensional growth of extravillous cytotrophoblasts promotes differentiation and invasion](#). *Placenta* 26(10): 709-720.
3. Warner, J. A., Zvezdaryk, K. J., Day, B., Sullivan, D. E., Pridjian, G. and Morris, C. A. (2012). [Human cytomegalovirus infection inhibits CXCL12- mediated migration and invasion of human extravillous cytotrophoblasts](#). *Virology* 9: 255.