Cell Invasion Assay Kit

Cat. No. ECM550

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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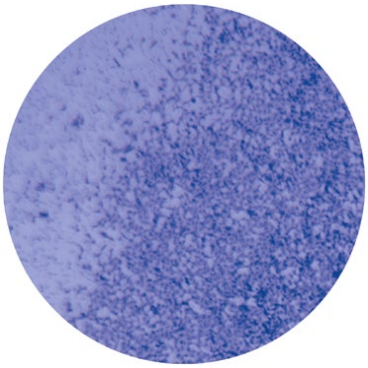
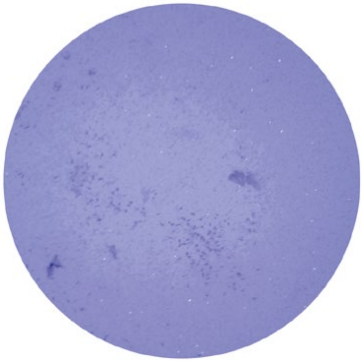
Introduction

Invasion through the extracellular matrix (ECM) is an important step in tumor metastasis. Cancer cells initiate invasion by adhering to and spreading along the blood vessel wall. Proteolytic enzymes, such as MMP collagenases, dissolve tiny holes in the sheath-like covering (basement membrane) surrounding the blood vessels to allow cancer cells to invade[1,2].

The CHEMICON Cell Invasion Assay Kit provides an efficient system for evaluating the invasion of tumor cells through a basement membrane model. The kit utilizes ECMatrix™, a reconstituted basement membrane matrix of proteins derived from the Engelbreth Holm-Swarm (EHS) mouse tumor[3]. We examined the kit's performance using human fibrosarcoma (HT-1080) and non-invasive fibroblasts (NIH3T3).



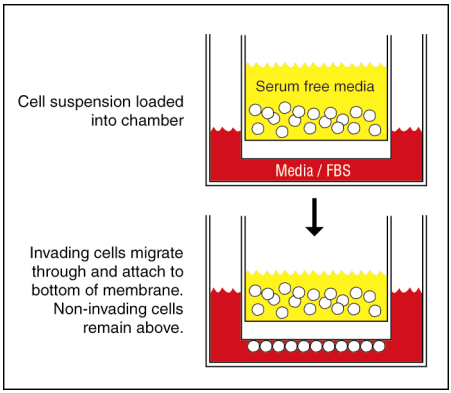
Invasion with HT-1080 cells



Non-Invasive NIH3T3 fibroblasts

Test Principle

The CHEMICON Cell Invasion Assay is performed in an Invasion Chamber, a 24-well tissue culture plate with 12 cell culture inserts. The inserts contain an 8 µm pore size polycarbonate membrane, over which a thin layer of ECMatrix™ is dried. The ECM layer occludes the membrane pores, blocking non-invasive cells from migrating through. Invasive cells, on the other hand, migrate through the ECM layer and cling to the bottom of the polycarbonate membrane.



Application

The CHEMICON Cell Invasion Assay Kit is ideal for evaluation of invasive tumor cells. Each CHEMICON Cell Invasion Assay Kit contains sufficient reagents for the evaluation of 12 samples.

The CHEMICON Cell Invasion Assay Kit is intended for research use only; not for diagnostic or therapeutic applications.

Kit Components

1. Invasion Chamber: (Part No. 70019) One 24-well plate with 12 ECMatrix™ layered cell culture inserts.
2. Cell Stain: (Part No. 20294) One 10 mL bottle.
3. Cotton Swabs: (Part No. 10202) 24 each.
4. Forceps: (Part No. 10203) One each.

Materials Not Supplied

1. Serum Free Media
2. Multichannel pipette and tips
3. Fetal Bovine serum
4. Tissue culture incubator
5. Microscope or Standard microplate reader
6. 10% Acetic acid
7. Beaker of Water
8. 96-well or other microtiter plate

Assay Instructions

NOTE: Steps 1-8 must be performed under sterile conditions.

1. Allow Invasion Chamber to adjust to room temperature in a tissue culture hood.
2. Sterilize forceps with 70% ethanol and handle inserts with forceps.
3. Add 300 µl of warm serum free media to the interior of the inserts. Allow this to rehydrate the ECM layer for 1-2 hours at room temperature.
4. Prepare a cell suspension containing 0.5-1.0 x 10^6 cells/ml in serum free media.
5. After rehydration from step 3, carefully remove media from the inserts without disturbing the membrane.
6. Add 500 µl of media containing 10% fetal bovine serum and/or chemoattractant to the lower chamber.
7. Add 300 µl of prepared cell suspension from step 4 to each insert.
8. Incubate 24-72 hours in a tissue culture incubator.
9. Using a cotton-tipped swab, gently remove non-invading cells as well as the ECMatrix gel from the interior of the inserts. Take care not to puncture the polycarbonate membrane. Be sure to remove cells on the inside perimeter, as any remaining cells inside the insert will contribute to background staining. Repeat procedure with a second clean cotton-tipped swab.
10. Add 500 µl of staining solution to the unoccupied wells of the plate.

11. Stain invasive cells on lower surface of the membrane by dipping inserts in the staining solution for 20 minutes.
12. Dip inserts in a beaker of water several times to rinse. Allow inserts to air dry.
13. Count cells by photographing the membrane through the microscope. Or alternatively, quantitate by dissolving stained cells in 10% acetic acid (100-200 µl/well) and transfer a consistant amount of the dye/solute mixture to a 96-well plate for colorimetric reading of OD at 560 nm.

Storage

Store kit materials at 2-8°C for up to 6 months. Do not freeze.

References

1. Albini, A., Iwamoto, Y., Kleinman, H.K., Martin, G.R., Aaronson, G.R., Kozlowski, J. M., and McEwan, R.N. (1987). A rapid in vitro assay for quantitating the invasive potential of tumor cells. Cancer Res. 47, 3239-3245
2. Repesh, L.A. (1989). A new in vitro assay for quantitating tumor cell invasion. Invasion Metastasis 9, 192-208
3. Terranova, V.P., Hujanen, E.S., Loeb, D.M., Martin, G.R., Thornburg, L., Glushko, V. (1986) Use of reconstituted basement membrane to measure cell invasiveness and select for highly invasive tumor cells. Proc. Natl. Acad. Sci. USA 83, 465
4. Liotta, L.A. (1984) Tumor invasion and metastasis: role of the basement membrane. Am. J. Pathol. 117, 339-348

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