ab325575 – Cell Migration and Invasion Assay (8 µm), Combo Kit

Utilizes basement membrane-coated inserts to assay the invasive properties of tumor cells.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab325575

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components.

Materials Supplied

Item	Quantity 2 x 12 Tests	Storage Condition
24-Well Migration Plate	1 unit	+4°C
24-Well Invasion Plate	1 unit	+4°C
Cell Stain Solution	20 mL	+4°C
Extraction Solution	20 mL	+4°C
Cotton Swabs	1 pack	+4°C
Forceps	1 unit	+4°C

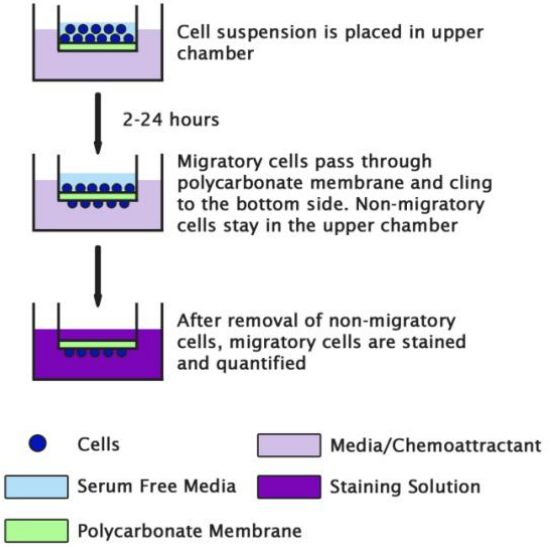
Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Migratory or invasive cell lines
- Cell culture medium
- Serum free medium, such as DMEM containing 0.5% BSA, 2 mM $CaCl_2$ and 2 mM $MgCl_2$
- Cell culture incubator (37°C, 5% CO_2 atmosphere)
- Light microscope
- 96-well microtiter plate

Cell Migration Assay Principle

The Cell Migration portion of this kit uses polycarbonate membrane inserts (8 µm pore size) in a 24-well plate. The membrane serves as a barrier to discriminate migratory cells from non-migratory cells. Migratory cells are able to extend protrusions towards chemo attractants (via actin cytoskeleton reorganization) and ultimately pass through the pores of the polycarbonate membrane. Finally, the cells are removed from the top of the membrane and the migratory cells are stained and quantified.

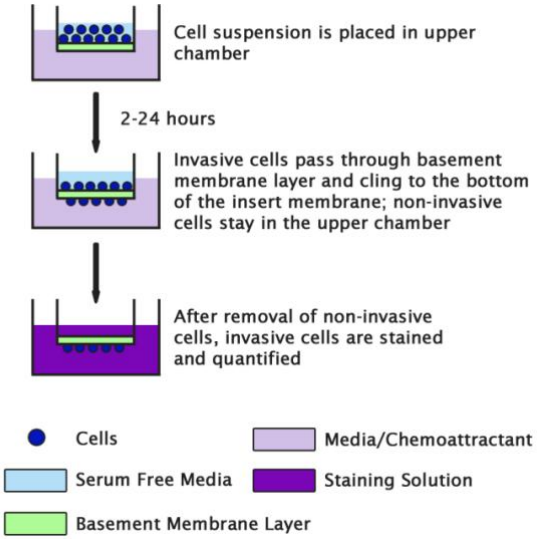


Cell Migration Assay Protocol

1. Under sterile conditions, allow the 24-well migration plate to warm up at room temperature for 10 minutes.
2. Prepare a cell suspension containing 0.5-1.0 x 10^6 cells/mL in serum free media. Agents that inhibit or stimulate cell migration can be added directly to the cell suspension.
   ∆***Note:*** *Overnight starvation may be performed prior to running the assay.*
3. Add 500 µL of media containing 10% fetal bovine serum or desired chemoattractant(s) to the lower well of the migration plate.
4. Add 300 µL of the cell suspension solution to the inside of each insert.
5. Incubate for 2-24 hours in a cell culture incubator.
6. Carefully aspirate the media from the inside of the insert. Wet the ends of 2-3 cotton-tipped swabs and gently swab the interior of the inserts to remove non-migratory cells. Take care not to puncture the polycarbonate membrane. Be sure to remove cells on the inside perimeter of the insert.
7. Transfer the insert to a clean well containing 400 µL of Cell Stain Solution and incubate for 10 minutes at room temperature.
8. Gently wash the stained inserts several times in a beaker of water. Allow the inserts to air dry.
9. **Optional:** Count migratory cells with a light microscope under high magnification objective, with at least three individual fields per insert.
10. Transfer each insert to an empty well, adding 200 µL of Extraction Solution per well, then incubating 10 minutes on an orbital shaker.
11. Transfer 100 µL from each sample to a 96-well microtiter plate and measure the OD 560nm in a plate reader.

Cell Invasion Assay Principle

The Cell Invasion Assay portion of this kit uses a 24-well plate containing polycarbonate membrane inserts (8 µm pore size); the upper surface of the insert membrane is coated with a uniform layer of dried basement membrane matrix solution. This basement membrane layer serves as a barrier to discriminate invasive cells from non-invasive cells. Invasive cells are able to degrade the matrix proteins in the layer, and ultimately pass through the pores of the polycarbonate membrane. Finally, the cells are removed from the top of the membrane and the invaded cells are stained and quantified.



Cell Invasion Assay Protocol

1. Under sterile conditions, allow the invasion chamber plate to warm up at room temperature for 10 minutes.
2. Rehydrate the basement membrane layer of the cell culture inserts by adding 300 µL of warm, serum-free media to the inner compartment. Incubate at room temperature for 1 hour.
3. Prepare a cell suspension containing 0.5-1.0 x 10^6 cells/mL in serum free media. Agents that inhibit or stimulate cell invasion can be added directly to the cell suspension.
   ∆***Note:*** *Overnight starvation may be performed prior to running the assay.*
4. Carefully remove the rehydration medium (step 2) from the inserts without disturbing the basement membrane layer.
   ∆***Note:*** *It will not affect the assay performance if a small amount of rehydration medium is left in the compartment.*
5. Add 500 µL of media containing 10% fetal bovine serum or desired chemoattractant(s) to the lower well of the invasion plate.
6. Add 300 µL of the cell suspension solution to the inside of each insert.
7. Incubate for 12-48 hours in a cell culture incubator.
8. Carefully aspirate the media from the inside of the insert. Wet the ends of 2-3 cotton-tipped swabs with water, flatten the ends of the swabs by pressing them against a clean hard surface, and gently swab the interior of the inserts to remove non-invasive cells. Take care not to puncture the polycarbonate membrane. Be sure to remove cells on the inside perimeter of the insert.
9. Transfer the insert to a clean well containing 400 µL of Cell Stain Solution and incubate for 10 minutes at room temperature.
10. Gently wash the stained inserts several times in a beaker of water. Allow the inserts to air dry.
11. **Optional:** Count invasive cells with a light microscope under high magnification objective, with at least three individual fields per insert.
12. Transfer each insert to an empty well, adding 200 µL of Extraction Solution per well, then incubating 10 minutes on an orbital shaker.
13. Transfer 100 µL from each sample to a 96-well microtiter plate and measure the OD 560nm in a plate reader.

Technical Support

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For all technical or commercial enquiries please go to:
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