

## ab325581 – Cell Invasion (Basement Membrane)-Fluorometric

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/ab325581](http://www.abcam.com/ab325581)

**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 12 Tests	Storage Condition
24-Well Invasion Plate	1 unit	+4°C
Forceps	1 unit	+4°C
Cell Detachment Solution	5 mL	+4°C
4X Lysis Solution	5 mL	+4°C
GR Dye	25 $\mu$ L	+4°C

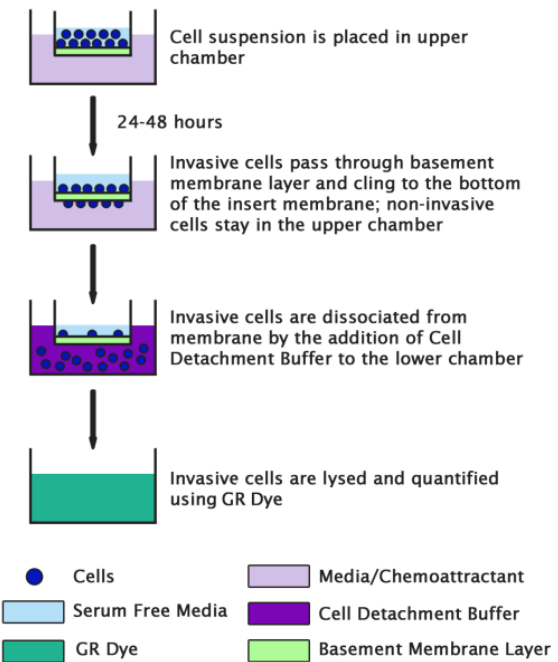
### Materials Required, Not Supplied

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

- Invasive cell lines
- Cell culture medium
- Serum free medium, such as DMEM containing 0.5% BSA, 2 mM CaCl<sub>2</sub> and 2 mM MgCl<sub>2</sub>
- Cell culture incubator (37°C, 5% CO<sub>2</sub> atmosphere)
- Light microscope
- 96-well microtiter plate
- Fluorescence plate reader

### Cell Invasion Assay Principle

The kit contains polycarbonate membrane inserts (8  $\mu$ m pore size) in a 24-well plate. 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, these cells are dissociated from the membrane and subsequently detected by the GR Dye.



### 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  $\mu$ L of warm, serum-free media to the inner compartment. Incubate at room temperature for 1 hour.  
 **$\Delta$ Note:** Overnight starvation may be performed prior to running the assay.
3. Prepare a cell suspension containing 0.5-1.0 x 10<sup>6</sup> cells/mL in serum free media. Agents that inhibit or stimulate cell invasion can be added directly to the cell suspension.
4. Carefully remove the rehydration medium (step 2) from the inserts without disturbing the basement membrane layer.  
 **$\Delta$ Note:** It will not affect the assay performance if a small amount of rehydration medium is left in the compartment.
5. Add 500  $\mu$ L of media containing 10% fetal bovine serum or desired chemoattractant(s) to the lower well of the invasion plate.
6. Add 300  $\mu$ L of the cell suspension solution to the inside of each insert.
7. Incubate for 24-48 hours in a cell culture incubator at 37°C in 5% CO<sub>2</sub> atmosphere.
8. Carefully aspirate the media from the inside of the insert. Transfer the insert to a clean well containing 225  $\mu$ L of Cell Detachment Solution. Incubate 30 minutes at 37°C.
9. Completely dislodge the cells from the underside of the membrane by gently tilting the insert several times in the detachment solution. Remove and discard the insert.

10. Prepare sufficient 4X Lysis Buffer/GR dye solution for all samples by diluting the dye 1:75 in 4X Lysis Buffer (for example, add 5  $\mu$ L dye to 370  $\mu$ L of 4X Lysis Buffer).
11. Add 75  $\mu$ L of 4X Lysis Buffer/GR dye solution to each well containing cells and 225  $\mu$ L of Cell Detachment Solution. Incubate 20 minutes at room temperature.
12. Transfer 200  $\mu$ L of the mixture to a 96-well plate suitable for fluorescence measurement. Read fluorescence with a fluorescence plate reader at 480nm/520nm.

### Technical Support

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