

Datasheet for ABIN2344867

CytoSelect™ 96-well Cell Invasion, Fluorometric

20 Publications



[Go to Product page](#)

Overview

Quantity:	96 tests
Reactivity:	Mammalian
Application:	Cellular Assay (CA)

Product Details

Brand:	CytoSelect™
Sample Type:	Serum, Cell Samples
Analytical Method:	Quantitative
Detection Method:	Fluorometric
Characteristics:	CytoSelect™ 96-well Cell Invasion Assay Kit utilizes basement membrane-coated inserts to assay the invasive properties of tumor cells. The kit does not require you to prelabel the cells with Calcein AM or remove non-invaded cells (i.e. cotton swabbing). Any invaded cells are first dissociated from the membrane, then lysed and detected with CyQuant® GR Dye. CytoSelect™ 96-well Cell Invasion Assay Kit provides a robust system for the quantitative determination of cell invasion. The kit contains sufficient reagents for the evaluation of 96 samples.
Components:	<ol style="list-style-type: none"> 1. 96-well ECM Invasion Plate : One sterile 96-well plate containing ECM-coated inserts (see Figure 1 for components) 2. 96-well Cell Harvesting Tray : One 96-well tray 3. Cell Detachment Solution : One 20 mL bottle 4. 4X Lysis Buffer : One 10 mL bottle 5. CyQuant® GR Dye : One 75 µL tube
Material not included:	1. Invasive cell lines

Product Details

2. Cell culture medium
3. Serum free medium, such as DMEM containing 0.5 % BSA, 2 mM CaCl₂ and 2 mM MgCl₂
4. Cell culture incubator (37 °C, 5 % CO₂ atmosphere)
5. Light microscope
6. 96-well microtiter plate
7. Microtiter plate reader 3 Top Plate Cover Middle Invasion Plate Membrane Chamber Bottom Feeder Tray : Components of the 96-well ECM Invasion Plate.

Target Details

Background: The ability of malignant tumor cells to invade normal surrounding tissue contributes in large part to the significant morbidity and mortality of cancers. Invasiveness requires several distinct cellular functions including adhesion, motility, detachment, and extracellular matrix proteolysis. Metastatic cells produce many proteolytic enzymes (e.g. lysosomal hydrolysates, collagenases, plasminogen activators) while the expression of certain cell surface protease receptors is also increased.

Application Details

Application Notes: Optimal working dilution should be determined by the investigator.

Comment:

- Fully quantify cell invasion with no manual cell counting
- Plate inserts are precoated with ECM basement membrane

Plate: Pre-coated

Protocol: The CytoSelect™ 96-well Cell Invasion Assay Kit contains polycarbonate membrane inserts (8 μm pore size) in a 96-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, the invaded cells are dissociated from the membrane and subsequently detected with CyQuant® GR Dye (Invitrogen).

Assay Procedure:

1. Under sterile conditions, allow the invasion plate to warm up at room temperature for 10 minutes.
2. Rehydrate the basement membrane layer of the membrane inserts by adding 100 μL of warm, serum-free media to the inner compartment. Incubate at room temperature for 1 hour.
3. Prepare a cell suspension containing 0.2-2.0 x 10⁶ 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

Application Details

- basement membrane layer. Note: It will not affect the assay performance if a small amount of rehydration medium is left in the compartment 4
5. Under sterile conditions, separate the cover and membrane chamber from the feeder tray. Add 150 μ L of media containing 10 % fetal bovine serum or desired chemoattractant(s) to the wells of the feeder tray.
 6. Place the membrane chamber back into the feeder tray (containing chemoattractant solution). Ensure no bubbles are trapped under the membrane.
 7. Gently mix the cell suspension from step 3 and add 100 μ L to the membrane chamber.
 8. Finally, cover the plate and transfer to a cell culture incubator for 12-24 hours.
 9. Just prior to the end of the incubation, pipette 150 μ L of prewarmed Cell Detachment Solution into wells of the clean, 96-Well Cell Harvesting Tray (provided).
 10. Carefully remove the 96-well Invasion Plate from the incubator. Separate the membrane chamber from the feeder tray.
 11. Remove the cells/media from the top side of the membrane chamber by aspirating or inverting. Place the membrane chamber into the Cell Harvesting Tray containing 150 μ L of Cell Detachment Solution (step 9). Incubate 30 minutes at 37 °C.
 12. Completely dislodge the cells from the underside of the membrane by gently tilting the membrane chamber several times in the Cell Detachment Solution.
 13. Prepare sufficient 4X Lysis Buffer/CyQuant® GR dye solution for all samples by diluting the dye 1:75 in 4X Lysis Buffer (for example, add 5 μ L dye to 370 μ L of 4X Lysis Buffer).
 14. Add 50 μ L of 4X Lysis Buffer/CyQuant® GR dye solution to each well (already containing 150 μ L of Cell Detachment Solution). Incubate 20 minutes at room temperature.
 15. Transfer 150 μ L of the mixture to a 96-well plate suitable for fluorescence measurement. Read the fluorescence with a fluorescence plate reader at 480 nm/520 nm. 5

Restrictions: For Research Use only

Handling

Storage: 4 °C

Storage Comment: Store all components at 4°C.

Publications

Product cited in: Pengjam, Madhyastha, Madhyastha, Yamaguchi, Nakajima, Maruyama: "Anthraquinone Glycoside Aloin Induces Osteogenic Initiation of MC3T3-E1 Cells: Involvement of MAPK Mediated Wnt and Bmp Signaling." in: **Biomolecules & therapeutics**, Vol. 24, Issue 2, pp. 123-31, (2016) ([PubMed](#)).

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