Datasheet for ABIN2344863

CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Fluorometric Format)

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Overview

Quantity:	12 tests
Reactivity:	Mammalian
Application:	Cellular Assay (CA)

Product Details

Brand:	CytoSelect™
Sample Type:	Serum, Cell Samples
Analytical Method:	Quantitative
Detection Method:	Fluorometric
Characteristics:	CytoSelect™ Cell Invasion Assay Kit utilizes basement membrane-coated inserts to assay the invasive properties of tumor cells. This Trial Size kit contains sufficient reagents for the evaluation of 4 samples.
Components:	1. ECM Invasion Chamber Plate : One 24-well plate containing 4 ECM-coated cell culture inserts. 2. Cell Detachment Solution : One 2 mL tube 3. 4X Lysis Buffer : One 2 mL tube 4. CyQuant® GR Dye : One 10 µL tube 5. Forceps : One each
Material not included:	1. Invasive cell lines 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

Product Details

7. Fluorescence plate reader

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™ Cell Invasion Assay Kit contains polycarbonate membrane inserts (8 µm 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 patented CyQuant® GR Dye (Invitrogen).
Assay Procedure:	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 106 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.

Application Details

7. Incubate for 24-48 hours at 37 °C in 5 % CO2 atmosphere.
8. Carefully aspirate the media from the inside of the insert. Transfer the insert to a clean well containing 225 µL 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/CyQuant® GR dye solution for all samples by dilutine the dye 1:75 in 4X Lysis Buffer (for example, add 5 µL dye to 370 µL of 4X Lysis Buffer).
11. Add 75 µL of 4X Lysis Buffer/CyQuant® GR dye solution to each well containing cells and 225 µL of Cell Detachment Solution. Incubate 20 minutes at room temperature.
12. Transfer 200 µL of the mixture to a 96-well plate suitable for fluorescence measurement. Read fluorescence with a fluorescence plate reader at 480nm/520nm.

Restrictions:	For Research Use only

Handling

Storage:	4 °C
Storage Comment:	Store all components at 4°C.

Publications

Product cited in:

Almami, Hegazy, Nabbi, Alshalalfa, Salman, Abou-Ouf, Riabowol, Bismar: "ING3 is associated with increased cell invasion and lethal outcome in ERG-negative prostate cancer patients." in: **Tumour biology**, (2016) (PubMed).

Engel, Ali, Adamus, Frank, Dad, Ali, Nebe, Atif, Ismail, Langer, Ahmad: "Antitumor evaluation of two selected Pakistani plant extracts on human bone and breast cancer cell lines." in: **BMC complementary and alternative medicine**, Vol. 16, pp. 244, (2016) (PubMed).

Osawa, Yokoyama, Shigeto, Futagami, Mizunuma: "Decreased expression of carbonyl reductase 1 promotes ovarian cancer growth and proliferation." in: **International journal of oncology**, Vol. 46, Issue 3, pp. 1252-8, (2015) (PubMed).