Product Manual

CytoSelect™ 96-Well Cell Migration and Invasion Assay (8 µm, Fluorometric Format)

Catalog Number

CBA-106-C	2 x 96 assays (96 migration + 96 invasion)

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Cell migration is a highly integrated, multistep process that orchestrates embryonic morphogenesis, tissue repair and regeneration. It plays a pivotal role in the disease progression of cancer, mental retardation, atherosclerosis, and arthritis. The initial response of a cell to a migration-promoting agent is to polarize and extend protrusions in the direction of the attractant; these protrusions can consist of large, broad lamellipodia or spike-like filopodia. In either case, these protrusions are driven by actin polymerization and can be stabilized by extracellular matrix (ECM) adhesion or cell-cell interactions.

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.

Cell Biolabs' CytoSelect™ 96-well Cell Migration and Invasion Assay Kit utilizes a polycarbonate membrane plate (8 µm pore size) or basement membrane-coated inserts to assay the migratory or invasive properties of cells. The kit does not require you to prelabel the cells with Calcein AM or remove non-migratory or non-invasive cells (i.e. cotton swabbing). Any migratory or invasive cells are first dissociated from the membrane, then lysed and detected with CyQuant® GR Dye.

The CytoSelect™ 96-well Cell Migration and Invasion Assay Kit provides a robust system for the quantitative determination of cell migration. Each assay contains sufficient reagents for the evaluation of 96 samples. The 8 µm pore size is optimal for epithelial and fibroblast cell migration. However, in the case of leukocyte chemotaxis, a smaller pore size (3 µm) is recommended.

Related Products

1. CBA-100: CytoSelect™ 24-Well Cell Migration Assay (8µm, Colorimetric)
2. CBA-101: CytoSelect™ 24-Well Cell Migration Assay (8µm, Fluorometric)
3. CBA-101-C: CytoSelect™ 24-Well Cell Migration and Invasion Assay (8µm, Fluorometric)
4. CBA-102: CytoSelect™ 24-Well Cell Migration Assay (5µm, Fluorometric)
5. CBA-103: CytoSelect™ 24-Well Cell Migration Assay (3µm, Fluorometric)
6. CBA-104: CytoSelect™ 96-Well Cell Migration Assay (3µm, Fluorometric)
7. CBA-105: CytoSelect™ 96-Well Cell Migration Assay (5µm, Fluorometric)
8. CBA-106: CytoSelect™ 96-Well Cell Migration Assay (8µm, Fluorometric)
9. CBA-112: CytoSelect™ 96-Well Cell Invasion Assay (Basement Membrane, Fluorometric)
10. CBA-120: CytoSelect™ 24-Well Wound Healing Assay (Light Microscopy)
11. CBA-125: Radius™ 24-Well Cell Migration Assay (Microscopy)
12. CBA-130: CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation)

Kit Components

1. 96-well Cell Migration Plate (Part No. 10601): One sterile 96-well plate (see Figure 1 for components)
2. 96-well Cell Invasion Plate (Part No. 11201): One sterile 96-well plate containing ECM-coated inserts (see Figure 1 for components)
3. 96-well Cell Harvesting Tray (Part No. 10402): Two 96-well trays
4. Cell Detachment Solution (Part No. 10403): Two 20 mL bottles
5. 4X Lysis Buffer (Part No. 10404): Two 10 mL bottles
6. CyQuant® GR Dye (Part No. 10105): Two 75 µL tubes

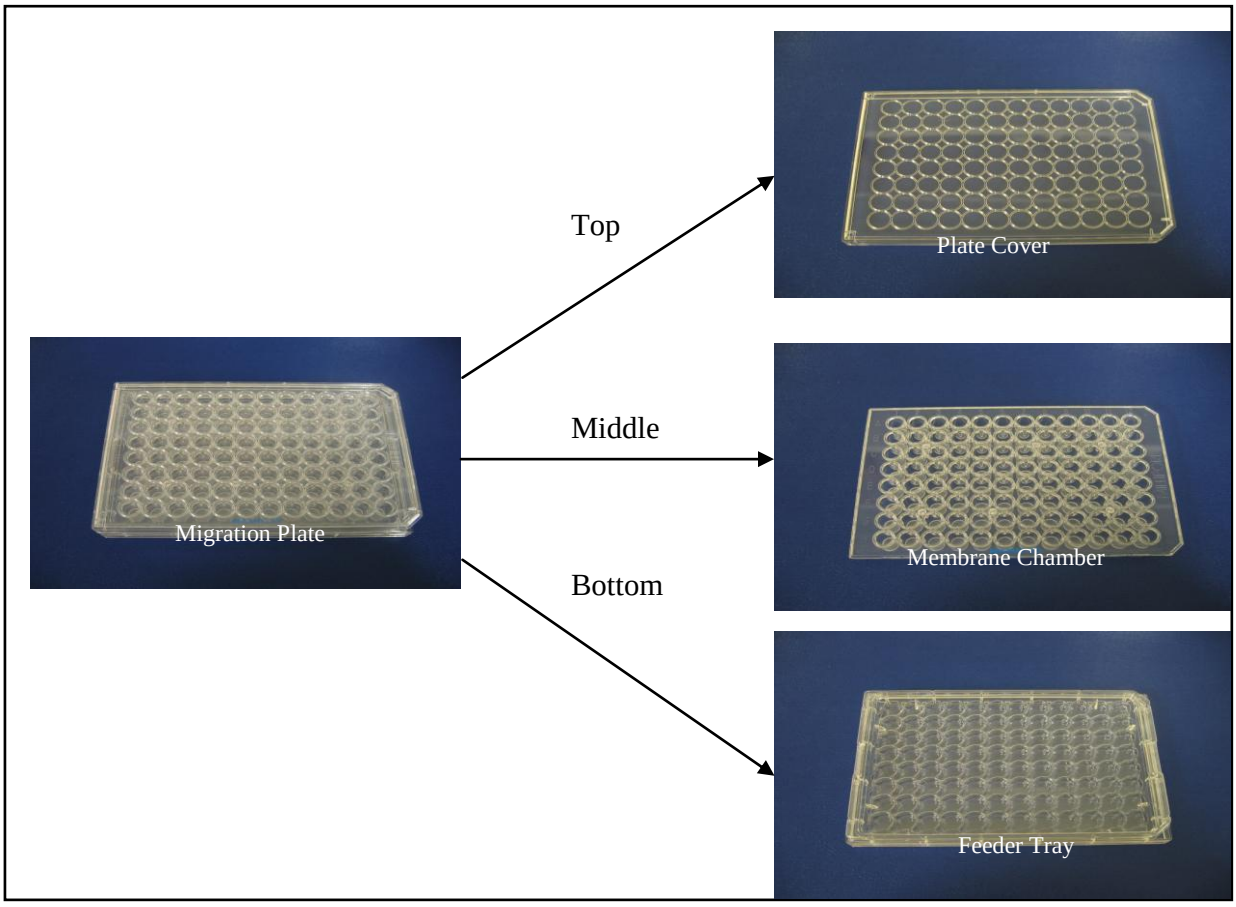


Figure 1: **Components of the 96-well Cell Migration Plate.**

Materials Not Supplied

1. Migratory cell lines
2. Cell culture medium

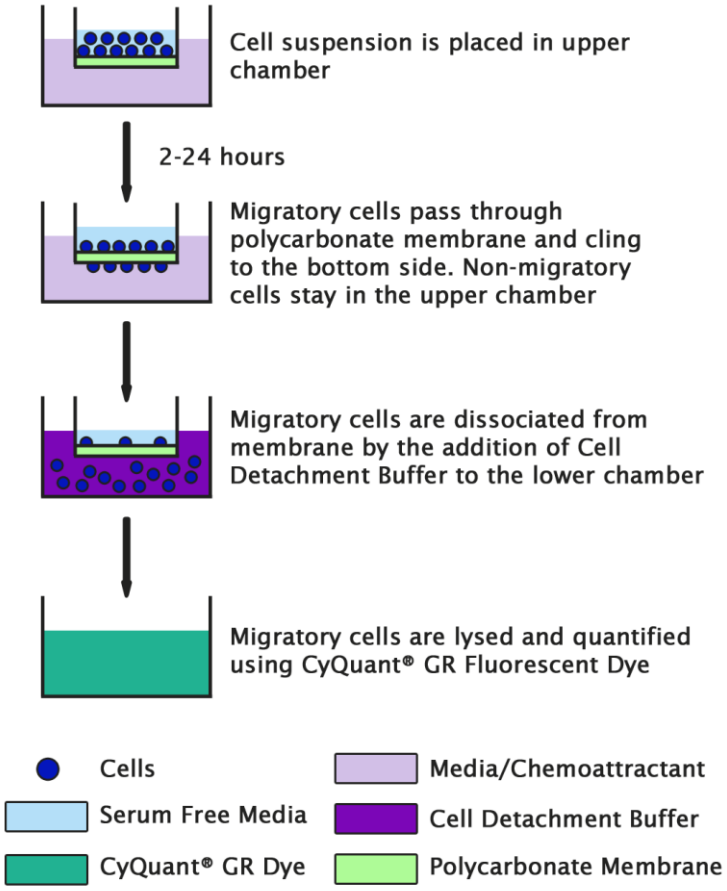
3. Serum free medium, such as DMEM containing 0.5% BSA, 2 mM $CaCl_2$ and 2 mM $MgCl_2$
4. FBS or desired chemoattractant
5. Cell culture incubator (37ºC, 5% CO_2 atmosphere)
6. Light microscope
7. 96-well plate suitable for a fluorescence plate reader
8. Fluorescence plate reader

Storage

Store all components at 4ºC until their expiration dates.

Cell Migration Assay Principle

The CytoSelect™ 96-well Cell Migration Assay Kit contains a polycarbonate membrane chamber (8 µm pore size) in a 96-well plate. The membrane serves as a barrier to discriminate migratory cells from non-migratory cells. Migratory cells are able to extend protrusions towards chemoattractants (via actin cytoskeleton reorganization) and ultimately pass through the pores of the polycarbonate membrane. These migratory cells are then dissociated from the membrane and subsequently detected with CyQuant® GR Dye.

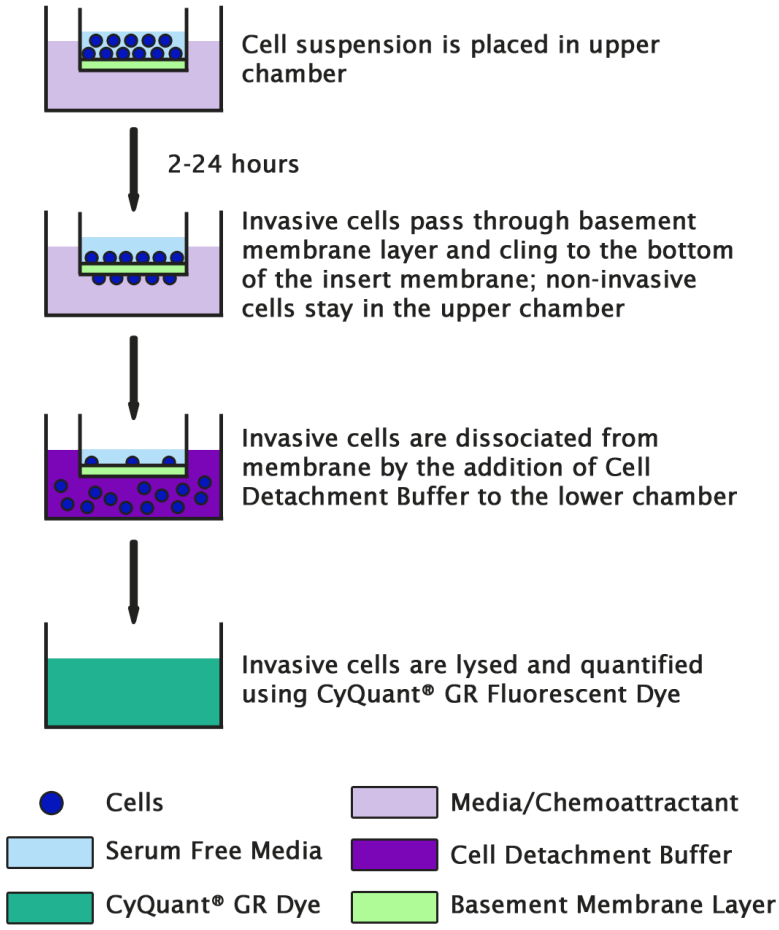


Cell Migration Assay Protocol

1. Allow the 96-well Migration Plate to warm up at room temperature for 10 minutes.
2. Prepare a cell suspension containing 0.1-1.0 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. Under sterile conditions, separate the cover and membrane chamber from the 96-well Migration Plate.
4. Add 150 µL of media containing 10% fetal bovine serum or desired chemoattractant(s) to the wells of the feeder tray.
5. Place the membrane chamber back into the feeder tray (containing chemoattractant solution). **Ensure no bubbles are trapped under the membrane.**
6. Gently mix the cell suspension (without chemoattractant) from step 2 and add 100 µL to the membrane chamber.
7. Finally, cover the plate and transfer to a cell culture incubator for 2-24 hours.
8. Just prior to the end of the incubation, pipette 150 µL of prewarmed Cell Detachment Solution into wells of the clean, 96-Well Cell Harvesting Tray (provided).
9. Carefully remove the 96-well Migration Plate from the incubator. Separate the membrane chamber from the feeder tray.
10. 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 µL of Cell Detachment Solution (step 8). Incubate 30 minutes at 37ºC.
11. Completely dislodge the cells from the underside of the membrane by gently tilting the membrane chamber several times in the Cell Detachment Solution.
12. 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 µL dye to 370 µL of 4X Lysis Buffer).
13. Add 50 µL of 4X Lysis Buffer/CyQuant® GR dye solution to each well (already containing 150 µL of Cell Detachment Solution). Incubate 20 minutes at room temperature.
14. Transfer 150 µL 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.

Cell Invasion Assay Principle

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.



Cell Invasion Assay Protocol

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 10^6 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.
   Note: It will not affect the assay performance if a small amount of rehydration medium is left in the compartment.
5. Under sterile conditions, separate the cover and membrane chamber from the feeder tray. Add 150 µL 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 µL 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 µL 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 µL of Cell Detachment Solution (step 9). Incubate 30 minutes at 37ºC.
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 µL dye to 370 µL of 4X Lysis Buffer).
14. Add 50 µL of 4X Lysis Buffer/CyQuant® GR dye solution to each well (already containing 150 µL of Cell Detachment Solution). Incubate 20 minutes at room temperature.
15. Transfer 150 µL 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.

Example of Results

The following figures demonstrate typical with the CytoSelect™ Cell Migration and Invasion Assay Kit. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 485/538 nm filter set and 530 nm cutoff. One should use the data below for reference only. This data should not be used to interpret actual results.

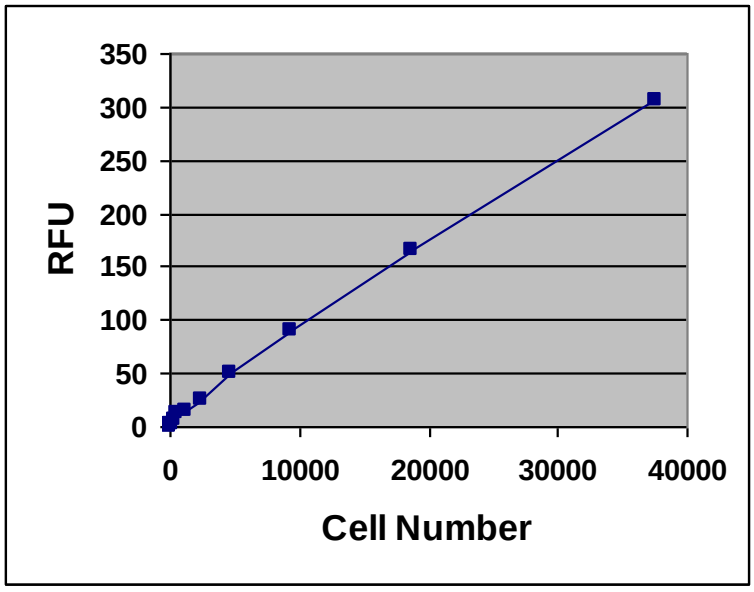


Figure 2: **Quantitation of Human HT-1080**. HT-1080 cells were titrated in Cell Detachment Buffer, then subsequently lysed and detected with 4X Lysis Buffer/Cyquant® GR Dye (150µL cell suspension was mixed with 50µL of 4X Lysis Buffer/dye).

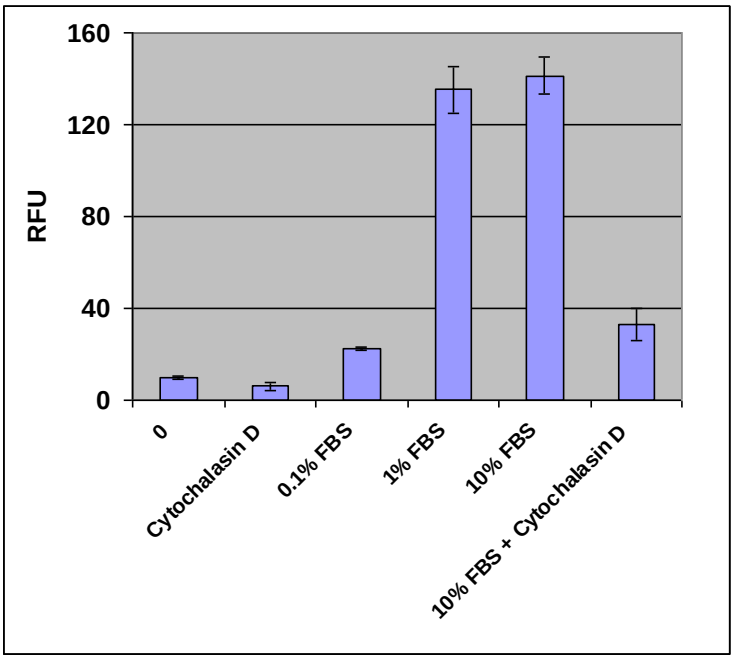


Figure 3: HT-1080 Chemotaxis. HT-1080 cells were allowed to migrate toward FBS for 4 hrs in the presence or absence of 2 µM Cytochalasin D, 30,000 cells were used in each assay. Migratory cells on the bottom of the polycarbonate membrane were detached and quantified by CyQuant® GR Dye as described in the Assay Protocol.

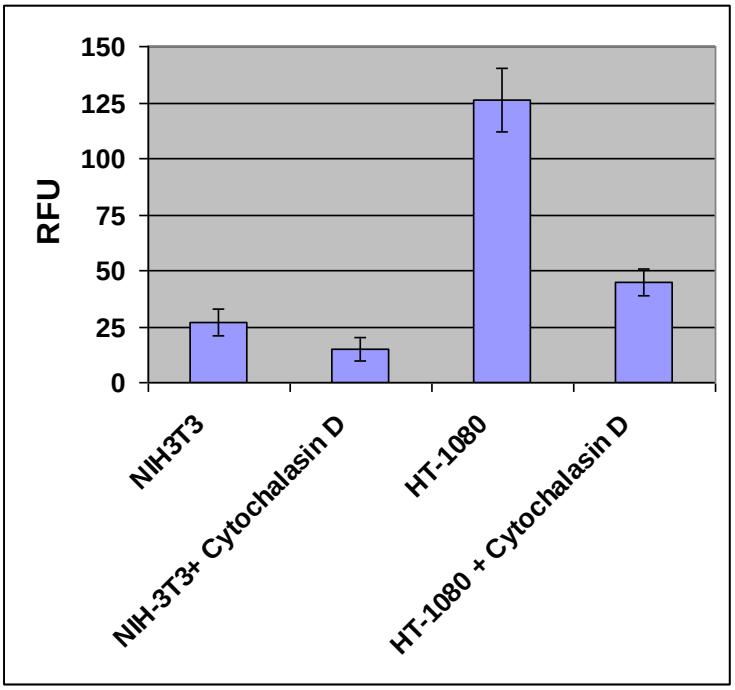


Figure 4: HT-1080 Invasion Assay. HT-1080 or NIH3T3 cells were allowed to invade toward 10% FBS for 24 hrs in the presence or absence of 2 µM Cytochalasin D, 200,000 cells were used in each assay. Invaded cells on the bottom of the polycarbonate membrane were stained (top) and quantified by CyQuant® GR Dye as described in Assay Protocol.

References

1. Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Borisy G, Parsons JT, Horwitz AR. (2003) *Science* **302**, 1704-9.
2. Horwitz R, Webb D. (2003) *Curr Biol.* **13**, R756-9.
3. Lauffenburger DA, Horwitz AF. (1996) *Cell* **84**, 359-369.

Recent Product Citations

1. Sharma, N. L. et al. (2014). The ETS family member GABPα modulates androgen receptor signalling and mediates an aggressive phenotype in prostate cancer. *Nucleic Acids Res*. **42**:6256-6269.
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4. Sharma, N.L. et al. (2014). The ETS family member GABPa modulates androgen receptor signaling and mediates an aggressive phenotype in prostate cancer. *Nucleic Acids Res.* 10.1093/nar/gku281.
5. Axlund, S.D. et al. (2010). HOXC8 inhibits androgen receptor signaling in human prostate cancer cells by inhibiting SRC-3 recruitment to direct androgen target genes. *Mol. Cancer Res.* **8**:1643-1655.
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7. Eckstein, N. et al. (2008). EGFR-pathway analysis identifies amphiregulin as a key factor for cisplatin resistance of human breast cancer cells. *J. Biol. Chem.* **283**:739-750.

License Information

CyQuant® GR Dye is licensed from Molecular Probes (Invitrogen).

Warranty

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