

---

Product Manual

# CytoSelect™ 24-Well Cell Migration and Invasion Assay (8 µm, Fluorometric Format)

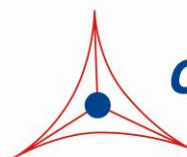
Catalog Number

CBA-101-C

2 x 12 assays (12 migration + 12 invasion)

FOR RESEARCH USE ONLY  
Not for use in diagnostic procedures

---



**CELL BIOLABS, INC.**  
*Creating Solutions for Life Science Research*

## **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, 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 (via transmembrane receptors).

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 hydrolyses, collagenases, plasminogen activators) while the expression of certain cell surface protease receptors is also increased.

Cell Biolabs' CytoSelect™ Cell Migration and Invasion Assay utilize polycarbonate membrane inserts (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 by the patented CyQuant® GR Dye (Invitrogen).

The CytoSelect™ Cell Migration and Invasive Assay Kit provides a robust system for the quantitative determination of cell migration. Each assay contains sufficient reagents for the evaluation of 12 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-101: CytoSelect™ 24-Well Cell Migration Assay (8µm, Fluorometric)
2. CBA-102: CytoSelect™ 24-Well Cell Migration Assay (5µm, Fluorometric)
3. CBA-103: CytoSelect™ 24-Well Cell Migration Assay (3µm, Fluorometric)
4. CBA-106: CytoSelect™ 96-Well Cell Migration Assay (8µm, Fluorometric)
5. CBA-111: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Fluorometric)

## **Kit Components**

1. 24-well Migration Plate (Part No. 10001): One 24-well plate containing 12 cell culture inserts (8 µm pore size)
2. Invasion Chamber Plate (Part No. 11001): One 24-well plate containing 12 ECM-coated cell culture inserts.
3. Cell Detachment Solution (Part No. 10403): One 20 mL bottle
4. 4X Lysis Buffer (Part No. 10404): One 10 mL bottle
5. CyQuant® GR Dye (Part No. 105101): One 50 µL tube
6. Forceps (Part No. 11005): One each

## Materials Not Supplied

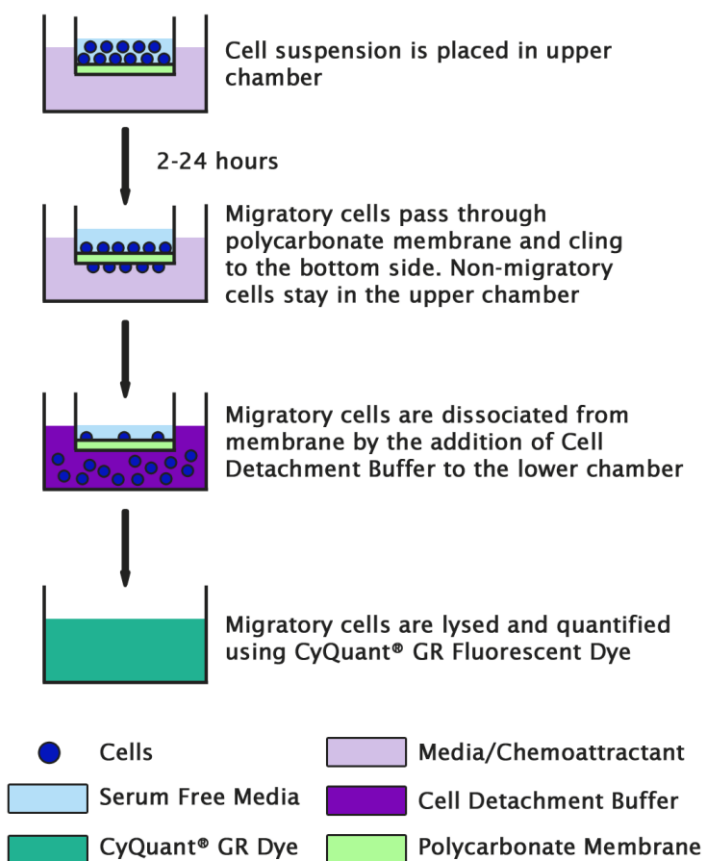
1. Migratory or invasive cell lines
2. Cell culture medium
3. Serum free medium, such as DMEM containing 0.5% BSA, 2 mM CaCl<sub>2</sub> and 2 mM MgCl<sub>2</sub>
4. Cell culture incubator (37°C, 5% CO<sub>2</sub> atmosphere)
5. Light microscope
6. 96-well plate suitable for a fluorescence plate reader
7. Fluorescence plate reader

## Storage

Store all components at 4°C.

## Cell Migration Assay Principle

The CytoSelect™ Cell Migration Assay Kit contains 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 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 by the patented CyQuant® GR Dye (Invitrogen).



## **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  $0.5-1.0 \times 10^6$  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  $\mu$ L of media containing 10% fetal bovine serum or desired chemoattractant(s) to the lower well of the migration plate.
4. Add 300  $\mu$ L 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. Transfer the insert to a clean well containing 225  $\mu$ L of Cell Detachment Solution. Incubate 30 minutes at 37°C.
7. 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.
8. 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  $\mu$ L dye to 370  $\mu$ L of 4X Lysis Buffer).
9. Add 75  $\mu$ L of 4X Lysis Buffer/CyQuant® GR dye solution to each well containing cells and 225  $\mu$ L of Cell Detachment Solution. Incubate 20 minutes at room temperature.
10. Transfer 200  $\mu$ L of the mixture a 96-well plate suitable for fluorescence measurement. Read fluorescence with a fluorescence plate reader at 480 nm/520 nm.

## **Cell Invasion Assay Principle**

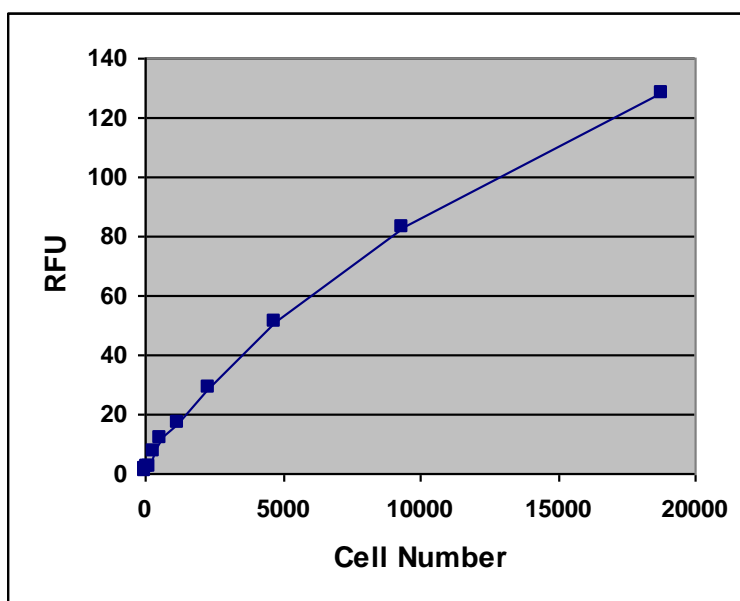
The CytoSelect™ Cell Invasion Assay Kit contains polycarbonate membrane inserts (8  $\mu$ m pore size) in a 24-well plate. The upper surface of the insert membrane is coated with a uniform layer of dried basement membrane matrix solution. This basement membrane layer serves as a barrier to discriminate 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, the invaded cells are dissociated from the membrane and subsequently detected with CyQuant® GR Dye.



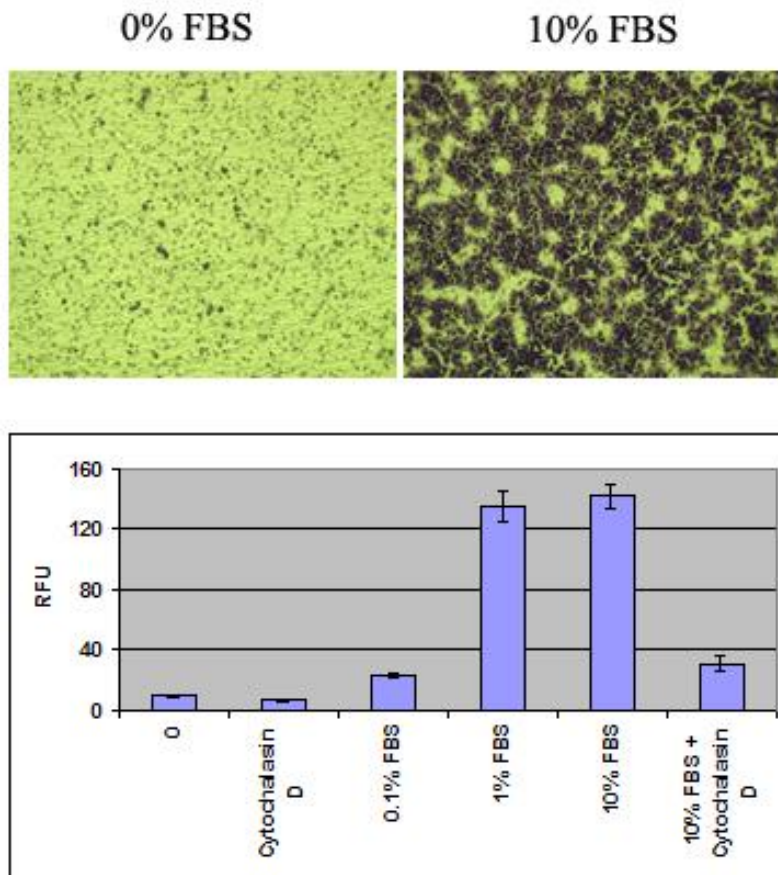
8. Carefully aspirate the media from the inside of the insert. Transfer the insert to a clean well containing 225  $\mu\text{L}$  of Cell Detachment Solution. Incubate 30 minutes at 37°C.
9. Completely dislodge the cells from the underside of the membrane by gently tilting the insert several times in the detachment solution. Remove and discard the insert.
10. Prepare sufficient 4X Lysis Buffer/CyQuant® GR dye solution for all samples by diluting the dye 1:75 in 4X Lysis Buffer (for example, add 5  $\mu\text{L}$  dye to 370  $\mu\text{L}$  of 4X Lysis Buffer).
11. Add 75  $\mu\text{L}$  of 4X Lysis Buffer/CyQuant® GR dye solution to each well containing cells and 225  $\mu\text{L}$  of Cell Detachment Solution. Incubate 20 minutes at room temperature.
12. Transfer 200  $\mu\text{L}$  of the mixture a 96-well plate suitable for fluorescence measurement. Read fluorescence with a fluorescence plate reader at 480 nm/520 nm.

### **Example of Results**

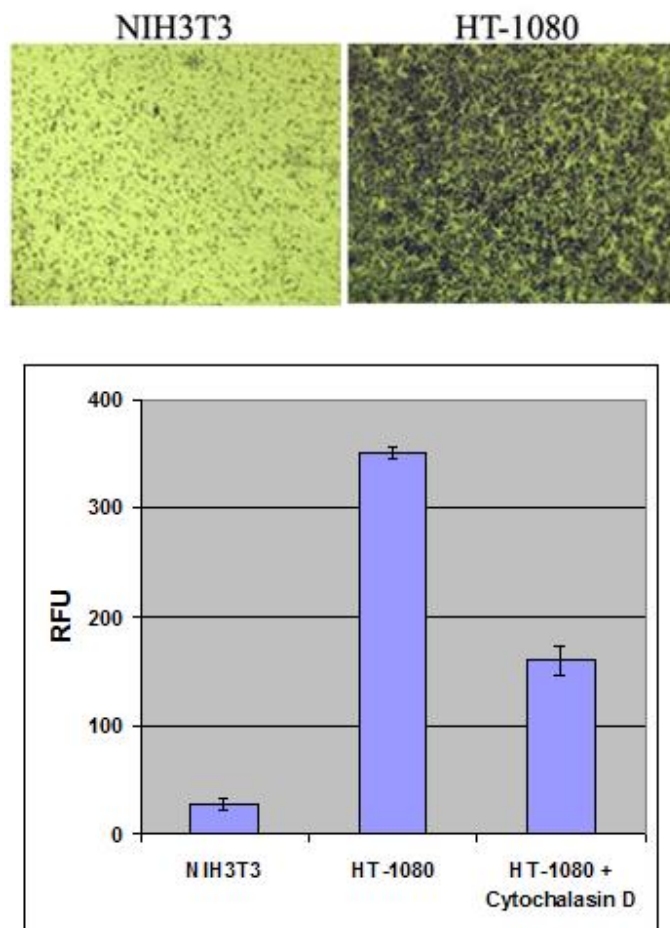
The following figures demonstrate typical with the CytoSelect™ Cell Migration and Invasion Assay Kit. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 485/538 nm filter set and 530 nm cutoff. One should use the data below for reference only. This data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



**Figure 1: 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 (150  $\mu\text{L}$  cell suspension was mixed with 50  $\mu\text{L}$  of 4X Lysis Buffer/dye).



**Figure 2. Human Fibrosarcoma HT-1080 Cell Migration.** HT-1080 was seeded at 30,000 cells/well and allowed to migrate toward FBS for 4 hrs in the presence or absence of 2  $\mu$ M Cytochalasin D. Migratory cells on the bottom of the polycarbonate membrane were stained (top panel picture) and quantified by CyQuant® GR Dye as described in the Assay Protocol.



**Figure 3. Human Fibrosarcoma HT-1080 Cell Invasion.** HT-1080 and NIH3T3 (negative control) were seeded at 300,000 cells/well and allowed to invade toward FBS for 24 hrs in the presence or absence of 2  $\mu$ M Cytochalasin D. Invasive cells on the bottom of the invasion membrane were stained (top panel picture) and quantified by CyQuant® GR Dye as described in Assay Protocol (bottom panel figure).

## 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.
4. Erkell, L. J., Schirmacher, V. (1988) *Cancer Res* **48**, 6933-6937.
5. Montgomery, A. M. P., De Clerck, Y. A., Langley, K. E., Reisfeld, R. A., Mueller, B. M. (1993) *Cancer Res* **53**,693-700.
6. Monsky, W. L., Lin, C. Y., Aoyama, A., Kelly, T., Akiyama, S. K., Mueller, S. C., Chen, W. T. (1994) *Cancer Res* **54**,5702-5710.



## **Recent Product Citations**

1. Khatiwada, P. et al. (2023). Peptides disrupting TM4SF3 interaction with AR or AR-V7 block prostate cancer cell proliferation. *Endocr Oncol*. doi: 10.1530/EO-23-0010.
2. Hara, T. et al. (2020). Cabozantinib inhibits AXL- and MET-dependent cancer cell migration induced by growth-arrest-specific 6 and hepatocyte growth factor. *Biochem Biophys Res*. doi: 10.1016/j.bbrep.2020.100726.
3. D'Almeida, O. et al. (2019). Encapsulated miR-200c and Nkx2.1 in a nuclear/mitochondria transcriptional regulatory network of non-metastatic and metastatic lung cancer cells. *BMC Cancer*. **19**(1):136. doi: 10.1186/s12885-019-5337-6.
4. Kathuria, H. et al. (2018). NKX2-1-AS1 negatively regulates CD274/PD-L1, cell-cell interaction genes, and limits human lung carcinoma cell migration. *Sci Rep*. **8**(1):14418. doi: 10.1038/s41598-018-32793-5.
5. She, S. et al. (2018). Proteomics Based Identification of Autotaxin As An Anti-Hepatitis B Virus Factor and a Promoter of Hepatoma Cell Invasion and Migration. *Cell Physiol Biochem*. **45**(2):744-760. doi: 10.1159/000487166.
6. Yamauchi, K. et al. (2017). 3,4',7-O-trimethylquercetin inhibits invasion and migration of ovarian cancer cells. *Anticancer Res*. **37**(6):2823-2829.
7. Fu, X. et al. (2016). Overexpression of BUB1B contributes to progression of prostate cancer and predicts poor outcome in patients with prostate cancer. *Onco Targets Ther*. **9**:221120.
8. Bhansali, M. et al. (2015). TM4SF3 and AR: A nuclear complex that stabilizes both proteins. *Mol Endocrinol*. doi:10.1210/me.2015-1075.
9. Lombard, A. P. et al. (2015). Dicer ablation promotes a mesenchymal and invasive phenotype in bladder cancer cells. *Oncol Rep*. **34**:1526-1532.
10. Liu, Y. et al. (2014). The role of von Willebrand factor as a biomarker of tumor development in hepatitis B virus-associated human hepatocellular carcinoma: A quantitative proteomic based study. *J Proteomics*. **106**:99-112.
11. Chavali, P. L. et al. (2014). TLX activates MMP-2, promotes self-renewal of tumor spheres in neuroblastoma and correlates with poor patient survival. *Cell Death Dis*. **5**:e1502.
12. Barui, S. et al. (2014). Simultaneous delivery of doxorubicin and curcumin encapsulated in liposomes of pegylated RGDK-lipopeptide to tumor vasculature. *Biomaterials*. **35**:1643-1656.
13. Peng, Y. et al. (2014). microRNA-503 inhibits gastric cancer cell growth and epithelial-to-mesenchymal transition. *Oncol Lett*. **7**:1233-1238.
14. Fatemi, M. et al. (2014). Epigenetic silencing of CHD5, a novel tumor-suppressor gene, occurs in early colorectal cancer stages. *Cancer*. **120**:172-180.
15. Saini, S. et al. (2014). Regulation of SRC kinases by microRNA-3607 located in a frequently deleted locus in prostate cancer. *Mol Cancer Ther*. **13**:1952-1963.
16. Kawamura, M. et al. (2014). The prognostic significance of eukaryotic elongation factor 1 alpha-2 in non-small cell lung cancer. *Anticancer Res*. **34**:651-658.
17. Uddin, M. et al. (2008). Marinobufagenin inhibits proliferation and migration of cytotrophoblast and CHO cells. *Placenta* **29**(3):266-273.
18. Gobeil, S. et al. (2008). A genome-wide shRNA screen identifies GAS1 as a novel melanoma metastasis suppressor gene. *Genes Dev*. **22**(21):2932-2940.

## **License Information**

This product is provided under an intellectual property license from Life Technologies Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased

product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The sale of this product is expressly conditioned on the buyer not using the product or its components, or any materials made using the product or its components, in any activity to generate revenue, which may include, but is not limited to use of the product or its components: (i) in manufacturing; (ii) to provide a service, information, or data in return for payment; (iii) for therapeutic, diagnostic or prophylactic purposes; or (iv) for resale, regardless of whether they are resold for use in research. For information on purchasing a license to this product for purposes other than as described above, contact Life Technologies Corporation, 5791 Van Allen Way, Carlsbad CA 92008 USA or [outlicensing@lifetech.com](mailto:outlicensing@lifetech.com).

## **Warranty**

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

## **Contact Information**

Cell Biolabs, Inc.  
7758 Arjons Drive  
San Diego, CA 92126  
Worldwide: +1 858-271-6500  
USA Toll-Free: 1-888-CBL-0505  
E-mail: [tech@cellbiolabs.com](mailto:tech@cellbiolabs.com)  
[www.cellbiolabs.com](http://www.cellbiolabs.com)

©2004-2024: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.