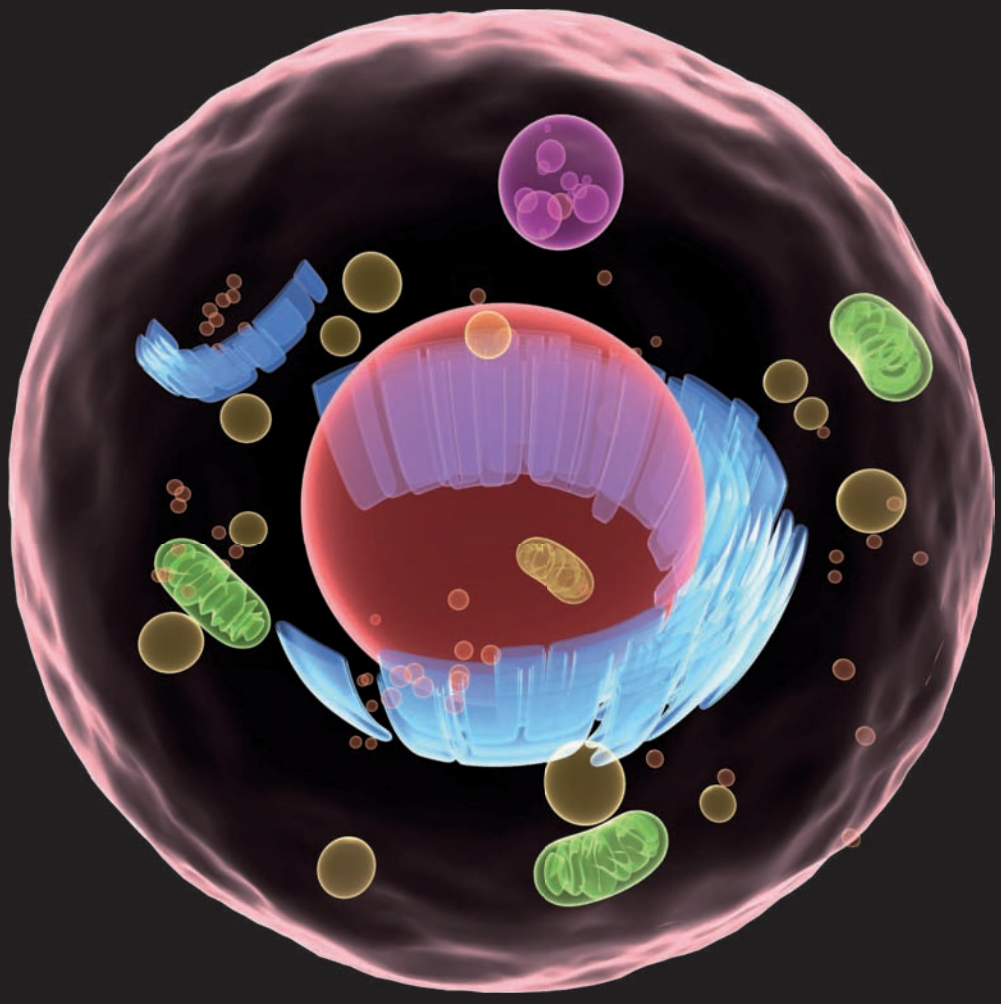
bioNova científica, s.l.

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

CELL-BASED ASSAYS



Tumor Cell / Soft Agar Assays

Transformation of normal cells into neoplastic cells results in a population capable of proliferating independently of internal and external signals that normally restrain growth. The soft agar colony formation assay has traditionally been used to monitor anchorage-independent growth, employing 3-4 weeks of cell growth followed by manual cell counting.

We have advanced the soft agar assay to eliminate tedious manual cell counting, allow high-throughput drug screening, and enable recovery of transformed cells for downstream analysis. These advances have also allowed us to develop a unique kit for the separation of clonogenic cancer cells from normal cells in heterogeneous solid tumors.

CytoSelect™ 96-Well Cell Transformation Assay—Traditional Soft Agar Colony Formation

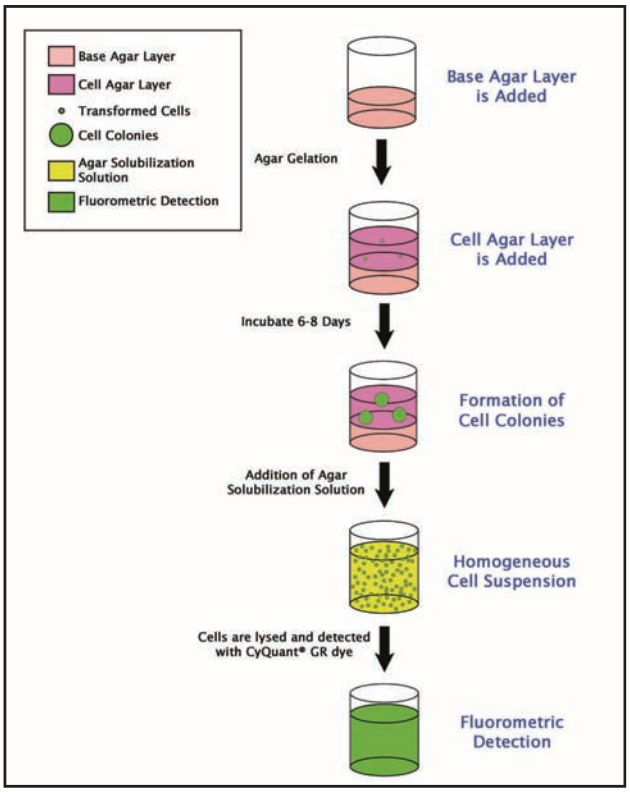
Our CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation) is suitable for measuring malignant transformation where no downstream analysis is required. Transformed cells cannot be recovered; however, no manual cell counting is required.

With this assay, cells are incubated in a semisolid agar medium for 6-8 days, then solubilized, lysed and detected using CyQuant® GR dye in a fluorometric plate reader.

Recent Product Citations

1. Inami, Y. et al. (2011). Persistent activation of Nrf2 through p62 in hepatocellular carcinoma cells. *J. Cell Biol.* **193(2):**275-284.
2. Carnahan, J. et al. (2010). Selective and potent Raf inhibitors paradoxically stimulate normal cell proliferation and tumor growth. *Mol. Cancer Ther*. **9**:2399-2410.
3. Liu, F. et al. (2010). Epigenomic alterations and gene expression profiles in respiratory epithelia exposed to cigarette smoke condensate. *Oncogene* **29**:3650-3664.
4. Iorns, E. et al. (2010). The role of SATB1 in breast cancer pathogenesis. *J. Natl. Cancer Inst.* **102(16):**1284-1296.
5. Faoro, L. et al. (2010). EphA2 mutation in lung squamous cell carcinoma promotes increased cell survival, cell invasion, focal adhesions, and mammalian target of rapamycin activation. *J. Biol. Chem.* **285**:18575-18585.
6. Rubio, R. et al. (2010). Deficiency in p53 but not retinoblastoma induced the transformation of mesenchymal stem cells in vitro and initiates leiomyosarcoma in vivo. *Cancer Res*. **70**:4185-4194.
7. Li, H. et al. (2009). Lysophosphatidic acid stimulates cell migration, invasion, and colony formation as well as tumorigenesis/metastasis of mouse ovarian cancer in immunocompetent mice. *Mol. Cancer Ther.* **8**:1692-1701.

- **Fast Results**: 6-8 days vs. 21 days
- **Plate Reader Convenience**: Eliminates manual counting
- **Versatile Format**: Designed for 96-well throughput, but can be adapted for 48, 24, 12 or 6-well

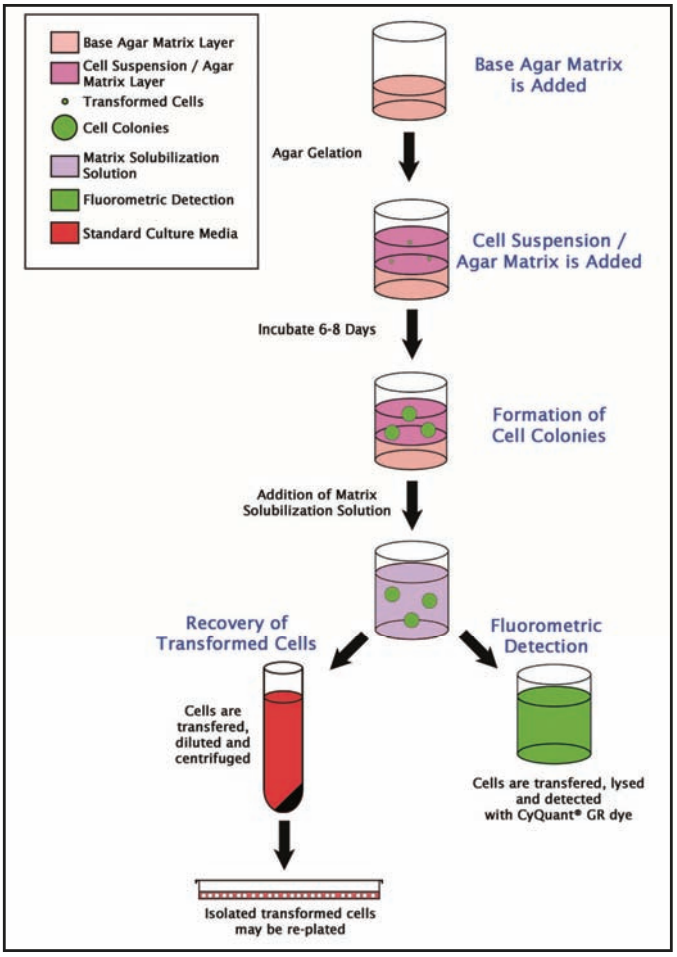


Cell Transformation Assay Principle.

Product Name	Detection	Size	Catalog Number
CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation)	Fluorometric	1 Plate*	CBA-130
		5 Plates*	CBA-130-5

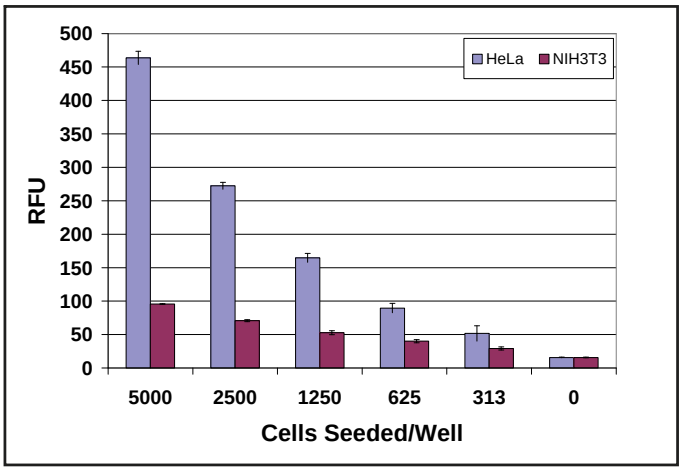
CytoSelect™ 96-Well Cell Transformation Assays—Advanced Soft Agar with Post-Incubation Cell Recovery

The CytoSelect™ 96-Well Cell Transformation Assay (Cell Recovery Compatible) provides a robust system for screening oncogenes and cell transformation inhibitors. Transformed cells may be recovered for further downstream analysis following colony formation.



Cell Transformation Assay Principle. Cell colonies form after a 6-8 day incubation with agar matrix. Transformed cells can then be either lysed and detected with a fluorescent dye or recovered and re-plated.

- **Faster Results**: 6-8 days vs. 21 days
- **Cell Recovery**: Transformed cells remain viable for further analysis
- **Plate Reader Convenience**: Eliminates manual counting of cells
- **Versatile Format**: Designed for 96-well throughput, but can be adapted for 48, 24, 12 or 6-well



Easy Fluorescence Detection with the CytoSelect™ Cell Transformation Assay. HeLa and NIH3T3 cells were seeded at various concentrations and cultured for 6 days. Transformed colonies were quantified according to the assay protocol.

Recent Product Citations

1. Mathew, B. et al. (2011). The novel role of the mu opioid receptor in lung cancer progression: A laboratory investigation. *Anesth. Analg.* **112**:558-567. (CBA-135)
2. Hirata, H. et al. (2010). Role of secreted Frizzled-related protein3 in human renal cell carcinoma. *Cancer Res.* **70**:1896-1905. (CBA-135)
3. Hirata, H. et al. (2009). Wnt antagonist gene DKK2 is epigenetically silenced and inhibits renal cancer progression through apoptotic and cell cycle pathways. *Clin. Cancer Res.* **15**:5678-5687. (CBA-135)
4. Xie, G. et al. (2009). Acetylcholine-induced activation of M3 muscarinic receptors stimulates robust matrix metalloproteinase gene expression in human colon cancer cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* **296:**G755-G763. (CBA-135)

Product Name	Detection	Size	Catalog Number
CytoSelect™ 96-Well Cell Transformation Assay (Cell Recovery Compatible)	Colorimetric	1 Plate*	CBA-135
		5 Plates*	CBA-135-5
	Fluorometric	1 Plate*	CBA-140
		5 Plates*	CBA-140-5
CytoSelect™ 384-Well Cell Transformation Assay**	Fluorometric	1 Plate***	CBA-145
		5 Plates***	CBA-145-5

*Each kit provides sufficient reagent quantities to perform 96, 48, 24, 12, or 6 tests in a 96, 48, 24, 12, or 6-well plate, respectively.
**The 384-well kit does not allow for cell recovery due to small well size.
***Each kit provides sufficient reagents for one or five 384-well plates respectively.

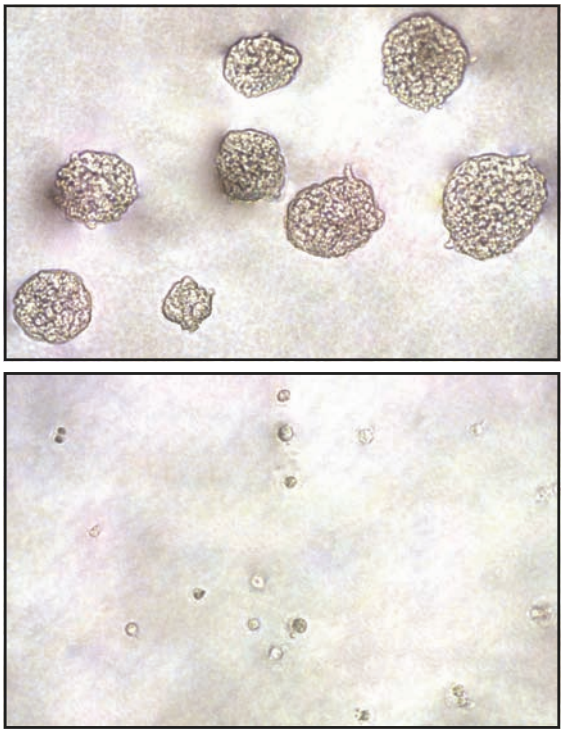
CytoSelect™ 96-Well *In Vitro* Tumor Sensitivity Assay

The CytoSelect™ *In Vitro* Tumor Sensitivity Assay provides a stringent, anchorage-independent model for chemosensitivity testing and possible anticancer drug screening. The assay uses a soft agar matrix to promote the colony formation of neoplastic cells in about a week. Cells are quantified using a standard ELISA plate reader.

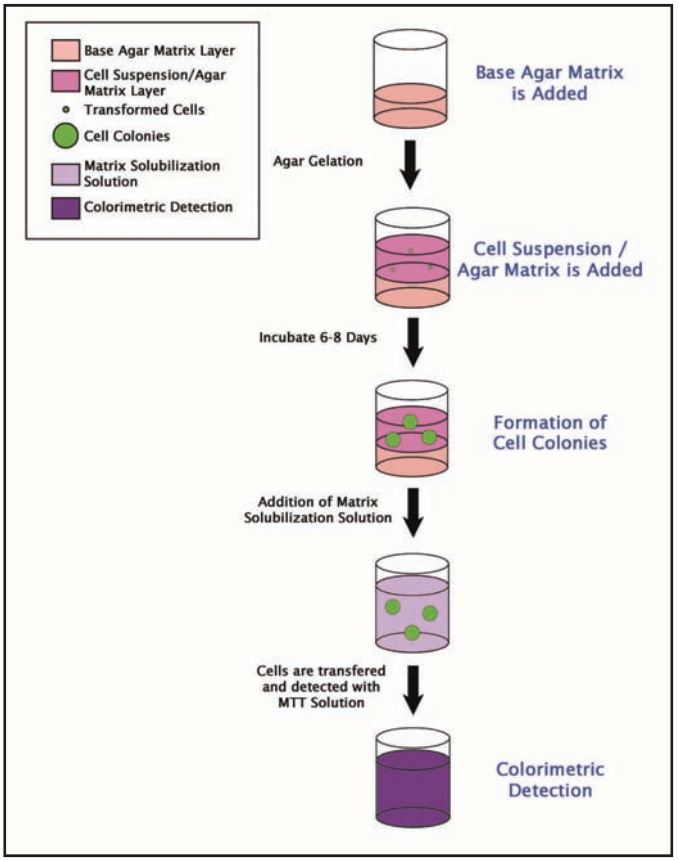
- **Fast Results**: 6-8 days
- ***In Vivo* Simulation**: Resembles a three-dimensional cell environment
- **Plate Reader Convenience**: Eliminates manual counting

Recent Product Citations

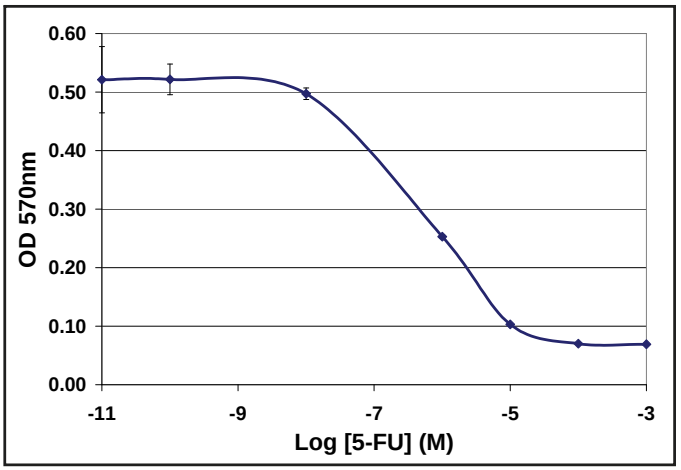
1. Itamochi, H. et al. (2011). Inhibiting the mTOR pathway synergistically enhances cytotoxicity in ovarian cancer cells induced by etoposide through upregulation of c-Jun. *Clin. Cancer Res.* **17**:4742-4750.
2. Kang, D.W. et al. (2010). Phospholipase D1 drives a positive feedback loop to reinforce the Wnt/ß-catenin/TCF signaling axis. *Cancer Res.* **70**:4233-4242.



Inhibition of HeLa Cell Anchorage-Independent Growth by Taxol. HeLa cells were cultured for 7 days in the absence (top) or presence (bottom) of 1 nM Taxol according to the assay protocol.



Tumor Sensitivity Assay Principle.



Inhibition of HeLa Cell Transformation by 5-Fluorouracil. HeLa cells were seeded at 5000 cells/well and cultured 7 days at various 5-FU concentrations. Cell transformation was determined according to the assay protocol. IC50 value of 5-Fluorouracil on HeLa cell anchorage-independent growth was determined to be ~ 1 µM.

Product Name	Detection	Size	Catalog Number
CytoSelect™ 96-Well *In Vitro* Tumor Sensitivity Assay	Colorimetric	96 Assays	CBA-150
		5 x 96 Assays	CBA-150-5

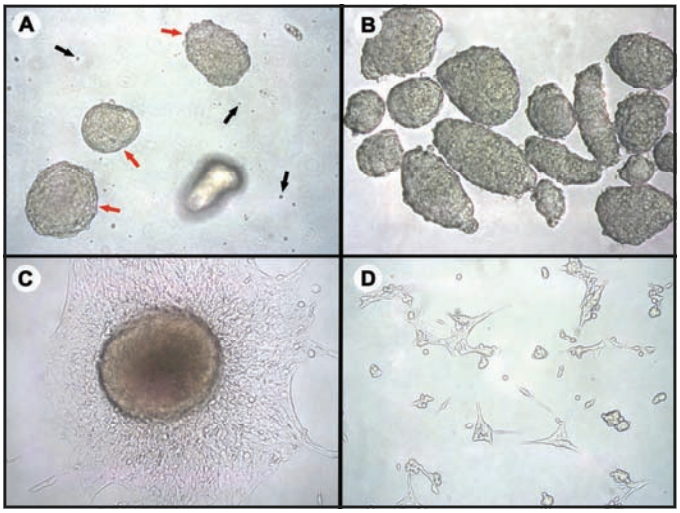
CytoSelect™ Clonogenic Tumor Cell Isolation Kit

Clean separation of clonogenic tumor cells from normal cells is critical for proper analysis of disease state progression. Due to the heterogeneity of many tumors, however, isolation of homogenous tumor cell populations can be difficult.

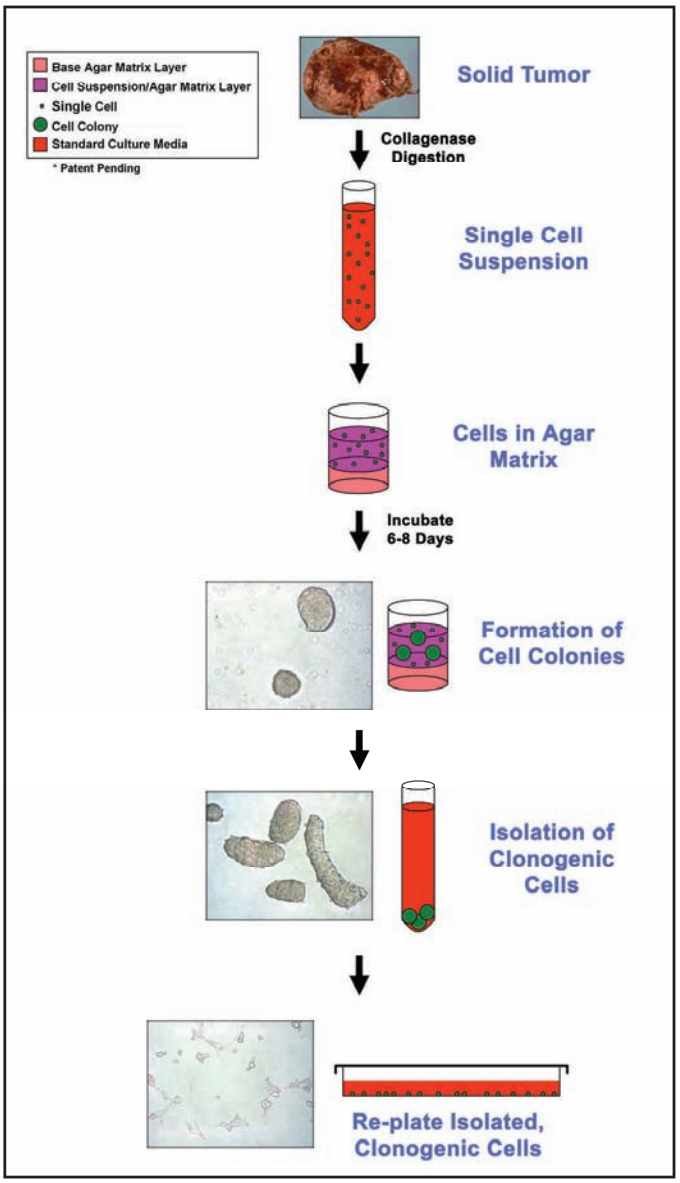
The CytoSelect™ Clonogenic Tumor Cell Isolation Kit uses a proprietary semisolid agar medium to facilitate colony formation by cells from solid tumors.

Colonies are grown in either a 6-well plate or a 35 mm dish. The colonies are then isolated away from single cells by size filtration.

- **Efficient**: Easily eliminates single cells from clonogenic tumor cell population
- **Versatile:** In addition to solid tumors, has potential use in isolating tumor stem cells



Clonogenic Colony Formation, Isolation and Re-plating. A: Clonogenic colony formation (red arrows) and single cells (black arrows) after 7 day incubation. **B**: Isolation of clonogenic colonies from single cells. **C**: Re-plated clonogenic colonies after 3 days (no trypsinization). **D**: Re-plated clonogenic colonies 1 day after trypsinization.



Clonogenic Tumor Cell Isolation Procedure.

Product Name	Size	Catalog Number
CytoSelect™ Clonogenic Tumor Cell Isolation Kit	5 Preps	CBA-155
	25 Preps	CBA-155-5

Cell Adhesion Assays

Cell adhesion is a complex mechanism involved in a variety of processes including cell migration/invasion, embryogenesis, wound healing and tissue remodeling. Cells can adhere to the ECM, forming complexes with cytoskeletal components, or to the endothelium.

Our CytoSelect™ Cell Adhesion Assays quantify adhesion of cells using a microplate reader or fluorometer; no manual cell counting is required.

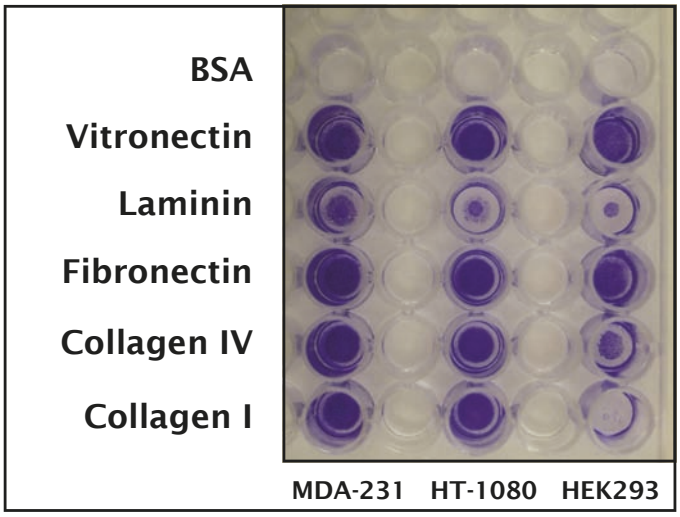
CytoSelect™ ECM Cell Adhesion Assays

The CytoSelect™ ECM Cell Adhesion Assays provide a quantitative method for evaluation of cell adhesion. The 48-well plate is precoated with your choice of substrate. Cells are seeded onto the substrate; adherent cells attach, while non-adherent cells are washed away. Adherent cells can be quantified on a standard plate reader or fluorometer.

- **Quantitative**: Measure results in a colorimetric or fluorescence plate reader
- **Flexible**: Uniform substrate layer of your choice of Collagen I, Collagen IV, Fibrinogen, Fibronectin, or Laminin; or choose the ECM array which contains all 5 ECM proteins

Recent Product Citations

1. Cervera, A.M. et al (2008). Cells silenced for SDHB expression display characteristic features of the tumor phenotype. *Cancer Res.* **68**:4058-4067. (CBA-050 and CBA-070)
2. Miao, H. et al. (2008). Gene expression and functional studies of the optic nerve head astrocyte transcriptome from normal African Americans and Caucasian Americans donors. *PLoS One* **3(8)**:E2847. (CBA-060)

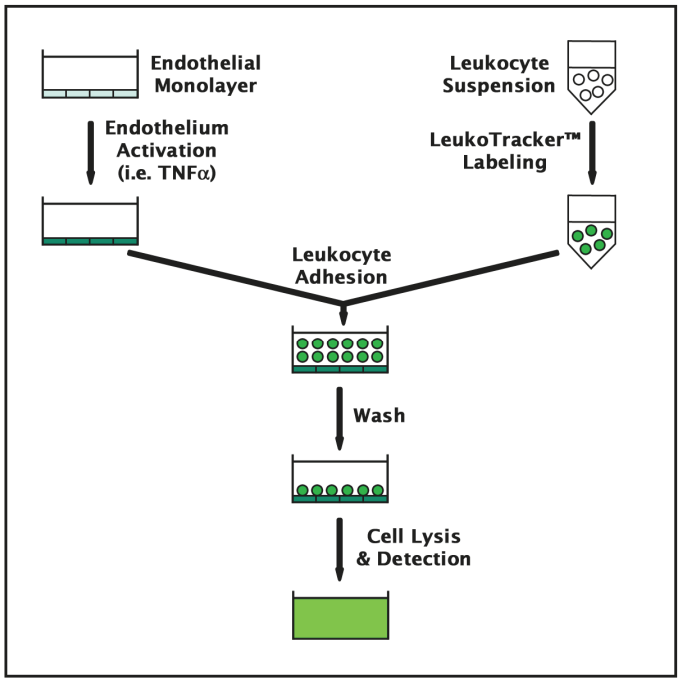


CytoSelect™ 48-well Cell Adhesion Assay. Serum starved cells from three different cell lines were allowed to attach to the ECM-coated 48-well plate for 1 hr at 100,000 cells/well. Adherent cells were stained according to the assay protocol.

Product Name	Detection	Size	Catalog Number
CytoSelect™ 48-Well Cell Adhesion Assay, ECM Array (Contains one row each of Collagen I, Collagen IV, Fibrinogen, Fibronectin, and Laminin)	Colorimetric	48 Assays	CBA-070
	Fluorometric	48 Assays	CBA-071
CytoSelect™ 48-Well Cell Adhesion Assay, Collagen I	Colorimetric	48 Assays	CBA-052
	Fluorometric	48 Assays	CBA-053
CytoSelect™ 48-Well Cell Adhesion Assay, Collagen IV	Colorimetric	48 Assays	CBA-060
	Fluorometric	48 Assays	CBA-061
CytoSelect™ 48-Well Cell Adhesion Assay, Fibrinogen	Colorimetric	48 Assays	CBA-058
	Fluorometric	48 Assays	CBA-059
CytoSelect™ 48-Well Cell Adhesion Assay, Fibronectin	Colorimetric	48 Assays	CBA-050
	Fluorometric	48 Assays	CBA-051
CytoSelect™ 48-Well Cell Adhesion Assay, Laminin	Colorimetric	48 Assays	CBA-056
	Fluorometric	48 Assays	CBA-057

CytoSelect™ Leukocyte Endothelium Adhesion Assays

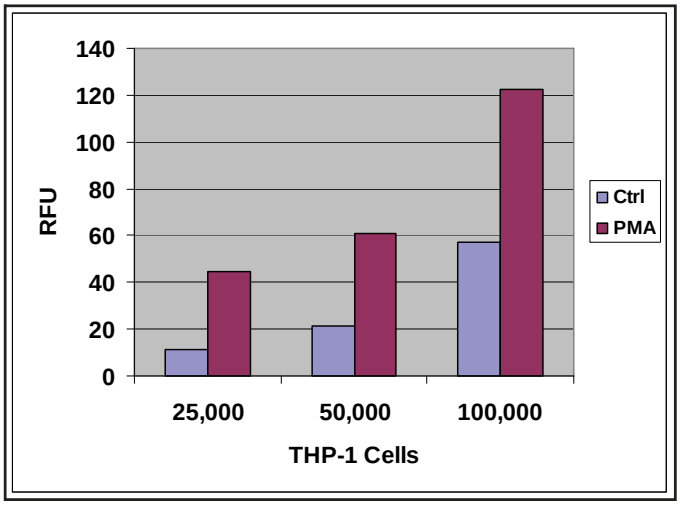
The CytoSelect™ Leukocyte Endothelium Adhesion Assays provide a robust system for the quantitative determination of interactions between leukocytes and endothelium. Adherent cells can be quantified on a fluorescence plate reader.



CytoSelect™ Leukocyte-endothelium Adhesion Assay Principle.

Recent Product Citations

1. Xue, J. et al. (2009). NFkB regulates thrombin-induced ICAM-1 gene expression in cooperation with NFAT by binding to the intronic NFkB site in the ICAM-1 gene. *Physiol. Genomics* **38**:42-53. (CBA-210)
2. Hernandez, L. et al. (2010). Activation of NF-kB signaling by inhibitor of NF-kB Kinase ß increases aggressiveness of ovarian cancer. *Cancer Res*. **70**:4005-4014. (CBA-215)
3. Gu, L. et al. (2010). Stat5 promotes metastatic behavior of human prostate cancer cells in vitro and in vivo. *Endocr. Relat. Cancer* **17**:481-493. (CBA-215)

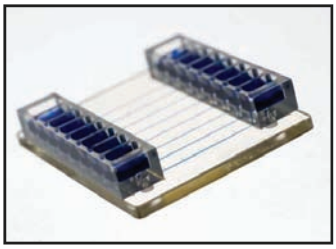


Human Monocytic THP-1 Adhesion to HUVEC Monolayer Using the CytoSelect™ Leukocyte-endothelium Adhesion Assay. HUVEC monolayer was treated with 1 µM PMA for 12 hrs. LeukoTracker™ labeled THP-1 cells were allowed to attach to HUVEC monolayer for 1 hr. Adherent cells were lysed and quantified as described in the assay protocol.

Product Name	Detection	Size	Catalog Number
CytoSelect™ Leukocyte-Endothelium Adhesion Assay	Fluorometric	96 Assays	CBA-210
CytoSelect™ Leukocyte-Epithelium Adhesion Assay	Fluorometric	96 Assays	CBA-211
CytoSelect™ Tumor-Endothelium Adhesion Assay	Fluorometric	96 Assays	CBA-215

CytoSelect™ Microfluidic Biochips

CytoSelect™ 8-Channel Microfluidic Biochips provide an environment that closely mimics in vivo shear stresses, resulting in more physiologically relevant cell adhesion data. The Biochips are self-contained units containing 8 channels with inlet/outlet ports at both ends of each channel. After adding cell samples, a syringe pump set to the proper flow rate applies the appropriate shear stress to the channel.



Product Name	Detection	Size	Catalog Number
CytoSelect™ 8-Channel ECM Microfluidic Biochips	Microscopy	2 Chips	CBA-003
		10 Chips	CBA-003-5
CytoSelect™ 8-Channel Endothelial Microfluidic Biochips	Microscopy	2 Chips	CBA-004
		10 Chips	CBA-004-5

Cell Migration & Invasion Assays

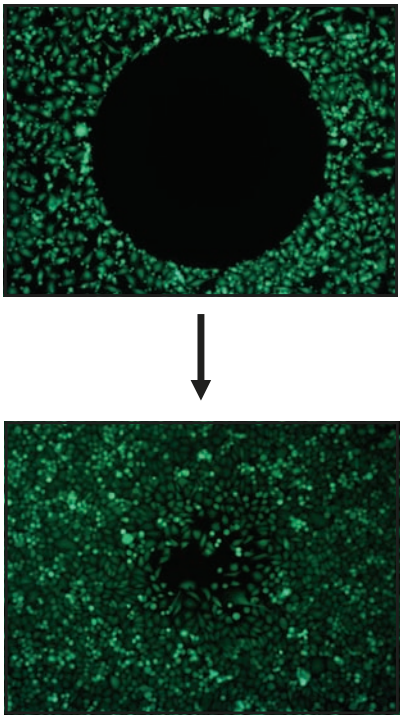
Cell migration and invasion are highly integrated, multi-step processes and play important roles in the progression of various diseases including cancer, atherosclerosis and arthritis.

Our cell migration assays are provided in two formats: 2-Dimensional Gap Closure and Boyden Chamber. Each format has its own advantages and applications. Use the information below to help choose the best format for your cell migration experimental goals.

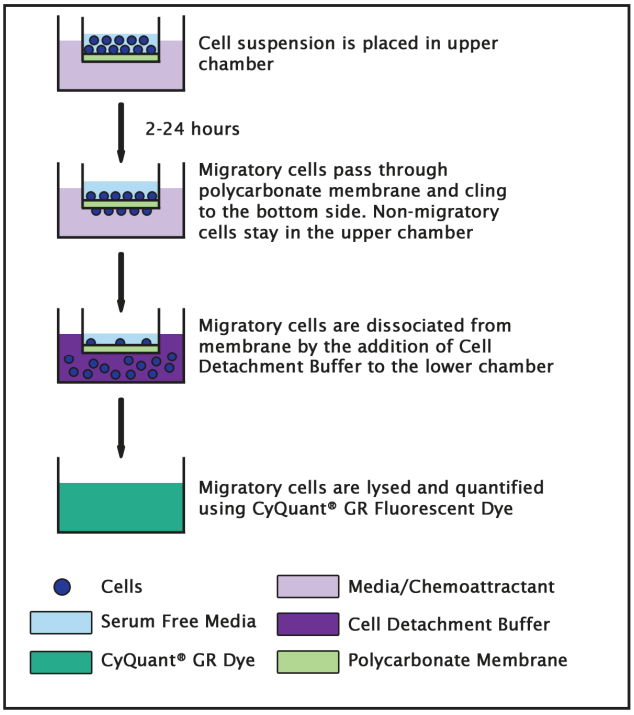
Cell invasion assays are provided in the Boyden Chamber format.

Cell Migration Format Selection Guide

	2D Gap Closure Assays (p. 8)	Boyden Chamber Assays (p. 9-14)
Type of Analysis	Qualitative or Quantitative	Quantitative
Detection Time	Endpoint or Real Time	Endpoint
Detection Method	Microscopy	Plate Reader
Chemoattractant Gradient	No	Yes
Relative Sensitivity	Good	Fair
Adaptability to Automation	Good	Poor
Cell Compatibility	Universal	Choose membrane pore size based on cell type/size



Example Results using 2D Gap Closure Assay.



Example of Boyden Chamber Assay Principle.

Radius™ Cell Migration Assays (2D Gap Closure)

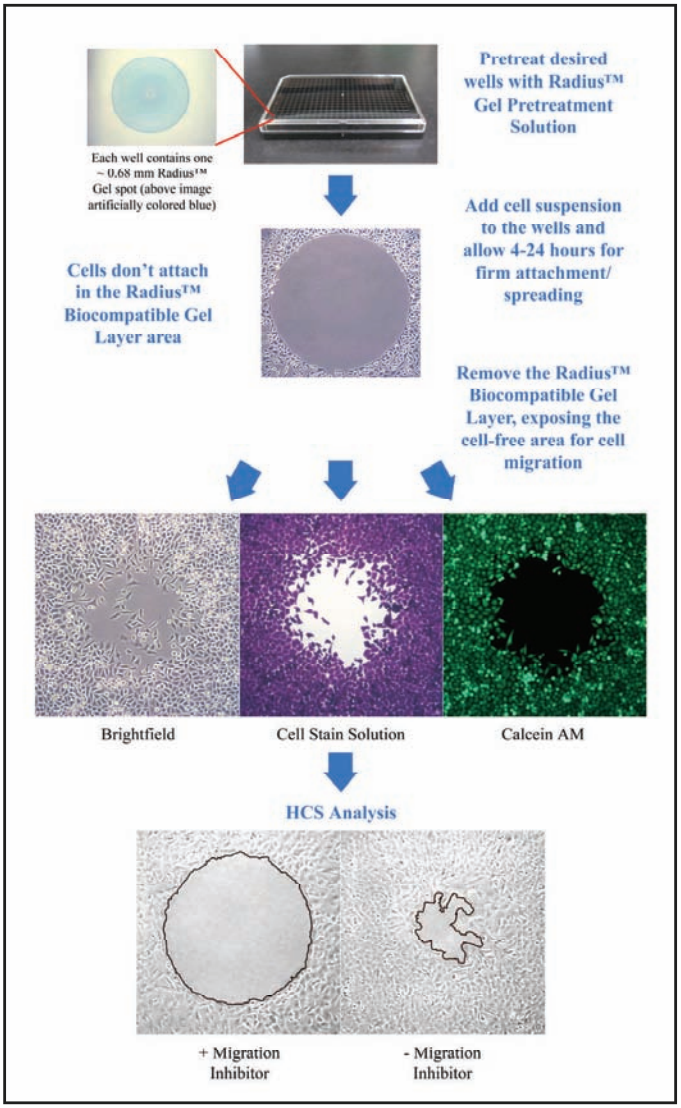
Radius™ Cell Migration Assays provide a unique alternative to the traditional Boyden Chamber migration assay. Radius assays allow you to measure cell migration at endpoint or in real time, and are ideal for time course migration studies.

Radius™ Cell Migration Assays use a cell culture plate containing a proprietary, carefully-defined biocompatible hydrogel (Radius™ gel) spot centralized at the bottom of each well. Cells seeded in the well will attach everywhere except on the Radius gel spot, creating a cell-free zone. Once cells attach, the Radius gel is removed and migration of cells across the cell-free zone begins. The gel removal step allows synchronization of a zero time point to facilitate well-to-well comparisons.

With Radius™ Cell Migration Assays, there are no cell culture inserts; so you don't need to worry about which pore size to choose for your cell type. Any adherent cell may be used in the assay.

Radius assays are supplied in 24-well, 96-well and 384-well formats. In addition, the 24-well assays are provided with your choice of coatings for proper cell attachment:

- Uncoated
- Collagen I-coated
- Fibronectin-coated
- Laminin-coated
- ECM Array with 6 wells of each of the above (uncoated, Collagen I, Fibronectin, Laminin); ideal if you are unsure which ECM protein may provide the best cell attachment



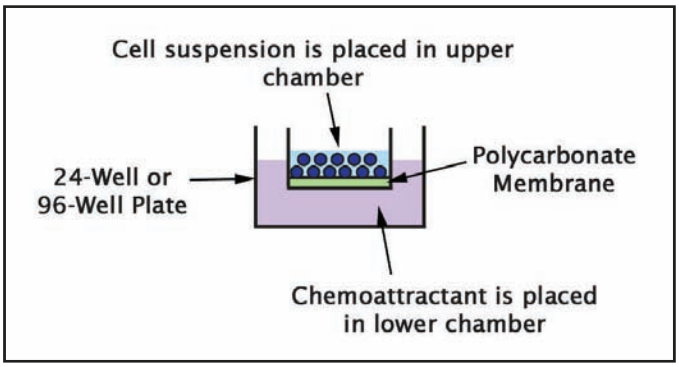
Assay Principle for the Radius™ Cell Migration Assays.

Product Name	Detection	Size	Catalog Number
Radius™ 24-Well Cell Migration Assay	Microscopy	24 Assays	CBA-125
		5 x 24 Assays	CBA-125-5
Radius™ 24-Well Cell Migration Assay (Collagen I Coated)	Microscopy	24 Assays	CBA-125-COL
Radius™ 24-Well Cell Migration Assay (Fibronectin Coated)	Microscopy	24 Assays	CBA-125-FN
Radius™ 24-Well Cell Migration Assay (Laminin Coated)	Microscopy	24 Assays	CBA-125-LN
Radius™ 24-Well Cell Migration Assay (ECM Array Coated)	Microscopy	24 Assays	CBA-125-ECM
Radius™ 96-Well Cell Migration Assay	Microscopy	96 Assays	CBA-126
		5 x 96 Assays	CBA-126-5
Radius™ 384-Well Cell Migration Assay	Microscopy	384 Assays	CBA-127
		5 x 384 Assays	CBA-127-5

CytoSelect™ Cell Migration and Invasion Assays (Boyden Chamber)

The Boyden Chamber has been extensively used and widely published as a tool for measuring cell migration and cell invasion in vitro. Our CytoSelect™ Cell Migration and Invasion Assays use this well-cited method to quantify cell migration and invasion with no manual cell counting required. Migratory or invasive cells are quantified using a colorimetric or fluorometric plate reader.

Cell migration may take on various forms and behaviors depending on the type and location of cells. Such subclasses of cell migration include chemotaxis, haptotaxis, and transmigration. Use the chart below to compare the various subclasses of cell migration as well as cell invasion, which will help you choose the assay best suited to your experimental goals.



Typical Well Setup for Boyden Chamber Assay.

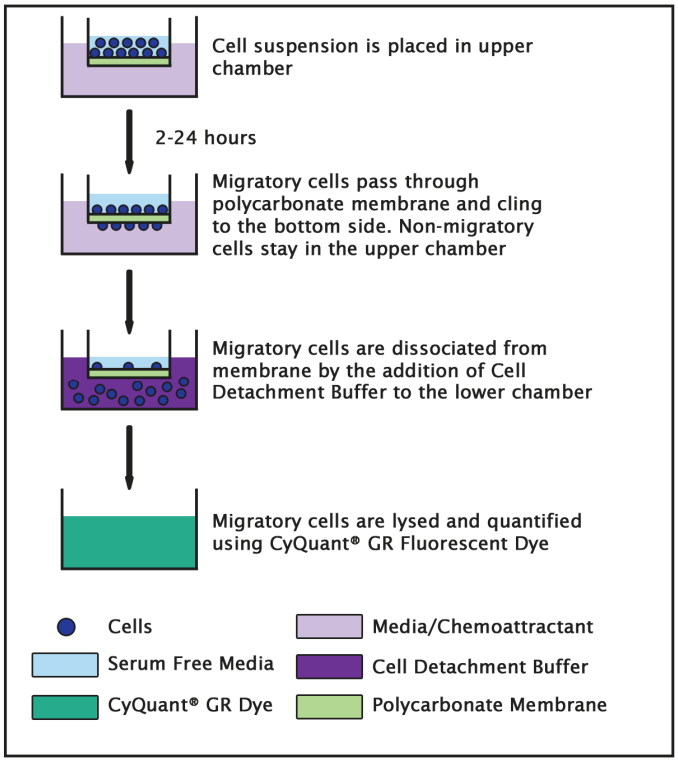
Boyden Chamber Assay Selection Guide

Assay	Definition	Cell Types	Pore Size	Insert Coating	Assay Formats
Chemotaxis (p. 10)	Migration of cells toward a chemoattractant (chemical signal) in the cell's surrounding environment	Neutrophils Leukocytes	3 µm	None	24-Well 96-Well
		Lymphocytes Monocytes Macrophages	5 µm	None	24-Well 96-Well
		Fibroblasts Endothelial Cells Epithelial Cells Tumor Cells	8 µm	None	24-Well 96-Well
		Astrocytes Slow-moving Cells	12 µm	None	24-Well
Haptotaxis (p. 11)	Migration of cells along a gradient of cellular adhesion sites or extracellular matrix-bound chemoattractants	Fibroblasts Endothelial Cells Epithelial Cells	8 µm	Collagen I (bottom)	24-Well
				Fibronectin (bottom)	24-Well
Transmigration (p. 12)	Migration of cells through the vascular endothelium toward a chemoattractant	Leukocytes	3 µm	None	24-Well
		Tumor Cells	8 µm	None	24-Well
Invasion (p. 13-14)	Movement of cells through the 3D extracellular matrix into neighboring tissues; includes ECM degradation and proteolysis	Fibroblasts Endothelial Cells Epithelial Cells Tumor Cells	8 µm	ECM Matrix (top)	24-Well 96-Well
				Collagen I (top)	24-Well 96-Well
				Laminin I (top)	24-Well 96-Well

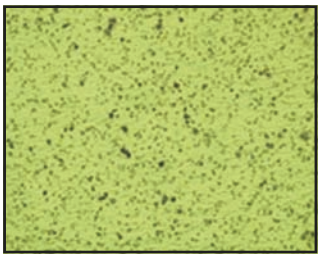
CytoSelect™ Cell Migration Assays—Chemotaxis

CytoSelect™ Cell Migration Assays are ideal for measuring chemotaxis. The kits utilize polycarbonate membrane inserts in 24-well or 96-well plates. Inserts are available with 4 different pore sizes to accommodate a variety of cell types.

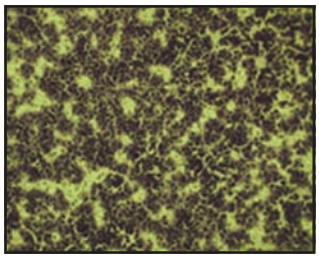
- **Fast Results**: Visualize chemotaxis in less than 6 hours with most cell types
- **Flexible**: Bottoms of membrane inserts are uncoated to allow use with any chemoattractant
- **Higher Throughput**: 96-well format available for fluorescence plate readers



Assay Principle for the CytoSelect™ Cell Migration Assay.



0% FBS



10% FBS

Migration of Human Fibrosarcoma HT-1080 Cells. Cells were seeded at 30,000 cells per well of a 24-well plate and allowed to migrate toward 10% FBS for 4 hours. Migratory cells were stained (above) and quantified in a fluorescence plate reader (data not shown).

Recent Product Citations

1. Tabata, C. et al. (2010). Novel clinical role of angiopoietin-1 in malignant pleural mesothelioma. *Eur. Respir. J.* **36(5)**:1099-1105. (CBA-100)
2. Awasthi, N. et al. (2009). Endothelial monocyte activating polypeptide II interferes with VEGF-induced proangiogenic signaling. *Laboratory Investigation* **89(1)**:38-46. (CBA-100)
3. Igarashi, J. et al. (2009). Transforming growth factor-ß1 down-regulates caveolin-1 expression and enhances sphingosine 1-phosphate signaling in cultured vascular endothelial cells. *Am. J. Physiol. Cell Physiol.* **297**:C1263-C1274. (CBA-100)
4. Izhak, L. et al. (2010). Predominant expression of CCL2 at the tumor site of prostate cancer patients directs a selective loss of immunological tolerance to CCL2 that could be amplified in a beneficial manner. *J. Immunol.* **184**:1092-1101. (CBA-101)
5. Shynlova, O. et al. (2008). Monocyte chemoattractant protein-1 (CCL-2) integrates mechanical and endocrine signals that mediate term and preterm labor. *J. Immunol.* **181**:1470-1479. (CBA-102)
6. Chatterjee, S. et al. (2009). Site-specific carboxypeptidase B1 tyrosine nitration and pathophysiological implications following its physical association with nitric oxide synthase-3 in experimental sepsis. *J. Immunol.* **183**:4055-4066. (CBA-104)
7. Christophi, G. et al. (2008). Modulation of macrophage infiltration and inflammatory activity by the phosphatase SHP-1 in virus-induced demyelinating disease. *J. Virol.* **83**:522-539. (CBA-105)
8. Saher, H. et al. (2010). Red wine consumption improves in vitro migration of endothelial progenitor cells in young healthy individuals. *Am. J. Clinical Nutrition* **92**:161-169. (CBA-106)

Product Name	Pore Size	Detection	Size	Catalog Number
CytoSelect™ 24-Well Cell Migration Assay	3 µm	Fluorometric	12 Assays	CBA-103
	5 µm	Fluorometric	12 Assays	CBA-102
	8 µm	Colorimetric	12 Assays	CBA-100
		Fluorometric	12 Assays	CBA-101
	12 µm	Colorimetric	12 Assays	CBA-107
		Fluorometric	12 Assays	CBA-108
CytoSelect™ 96-Well Cell Migration Assay	3 µm	Fluorometric	96 Assays	CBA-104
	5 µm	Fluorometric	96 Assays	CBA-105
	8 µm	Fluorometric	96 Assays	CBA-106

CytoSelect™ Cell Migration Assays—Haptotaxis

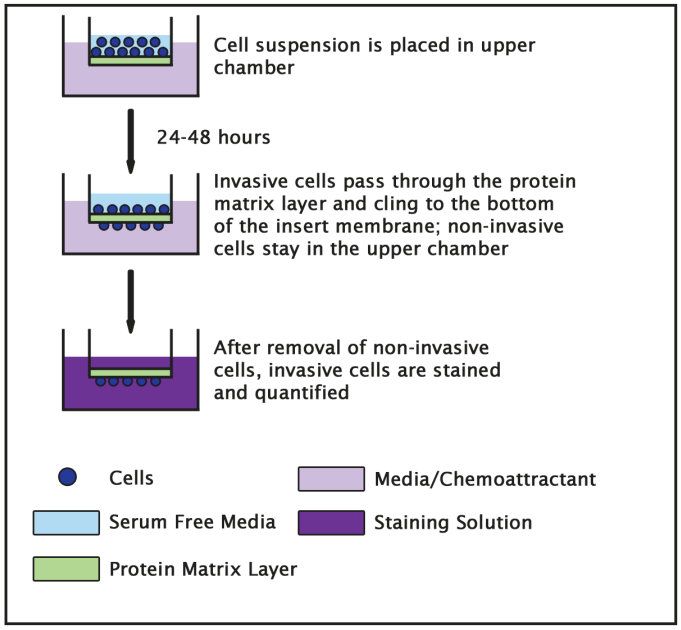
Haptotaxis describes the migration of cells toward a gradient of immobilized extracellular matrix. The CytoSelect™ Cell Haptotaxis Assays are ideal for determining the migratory properties of cells. The kits utilize polycarbonate membrane inserts with an 8 µm pore size in a 24-well plate.

The undersides of the inserts are coated with either Collagen or Fibronectin. The 8 µm pore size in the membrane inserts is ideal for epithelial cells, endothelial cells, fibroblasts, and other cells of similar size. The membrane serves as a barrier that allows discrimination of migratory cells from non-migratory cells.

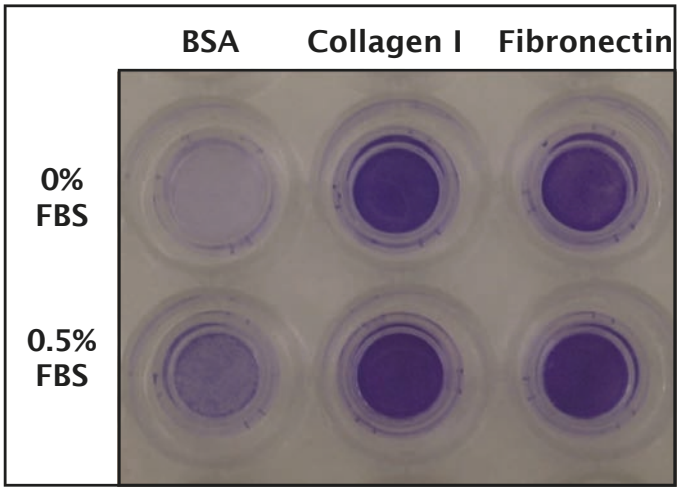
Recent Product Citation
Kamiya, K. et al. (2007). Protein Kinase C delta activated adhesion regulates vascular smooth muscle cell migration. J. Surg. Res. 141:91-96. (CBA-100-COL)

- **Fast Results**: Visualize cell haptotaxis in less than 6 hours with most cell types
- **Convenient**: Membrane inserts pre-coated on the underside with either Collagen I or Fibronectin
- **Versatile**: Useful with a variety of cell types including epithelial cells, endothelial cells, and fibroblasts*

*For leukocyte migration a 3 µm pore size is recommended. See our CytoSelect™ Cell Migration Assays (previous page) or the Leukocyte Transmigration Assay (next page).



Assay Principle for the CytoSelect™ Cell Haptotaxis Assay.



CytoSelect™ 24-well Cell Haptotaxis Assay. MDA-231 cells were seeded at 150,000 cells/well and allowed to migrate toward FBS for 4 hrs. Migratory cells, found on the bottom of the migration membrane, were stained according to the assay protocol.

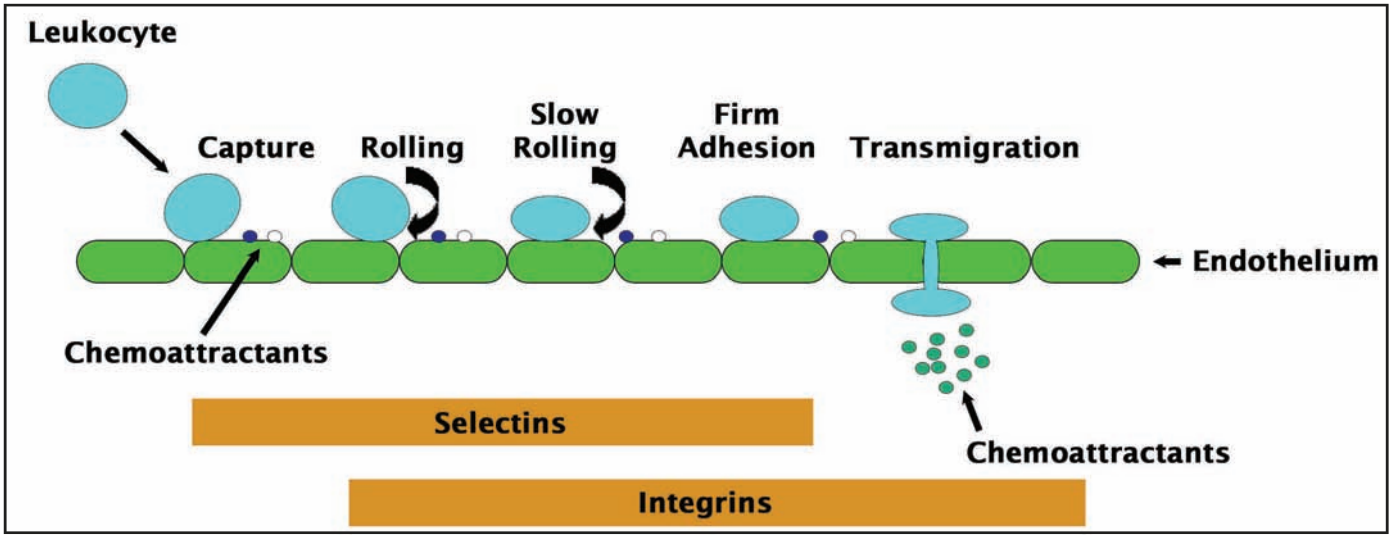
Product Name	Detection	Size	Catalog Number
CytoSelect™ 24-Well Cell Haptotaxis Assay, Collagen I-coated	Colorimetric	12 Assays	CBA-100-COL
	Fluorometric	12 Assays	CBA-101-COL
CytoSelect™ 24-Well Cell Haptotaxis Assay, Fibronectin-coated	Colorimetric	12 Assays	CBA-100-FN
	Fluorometric	12 Assays	CBA-101-FN

CytoSelect™ Cell Migration Assays—Transmigration

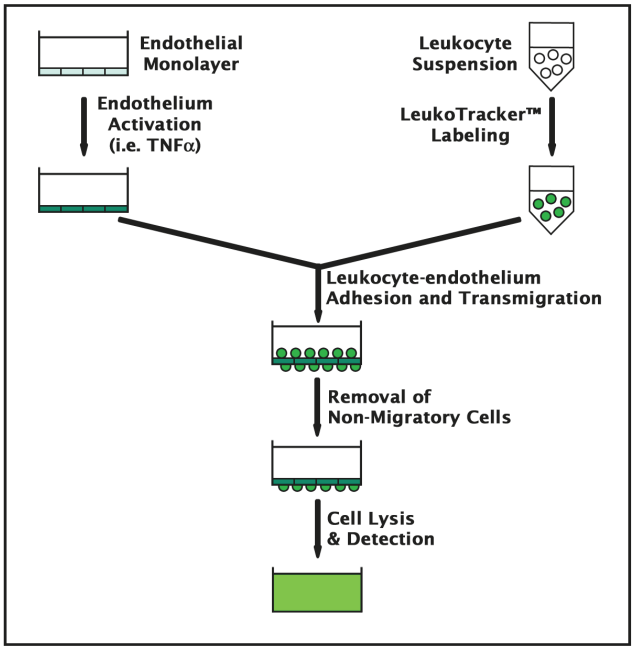
Cancer cell transmigration, particularly extravasation, is an important step in cancer metastasis. The CytoSelect™ Cell Transmigration Assays provide a robust system for the quantitation of transmigrations and interactions between endothelium and cancer cells. Migratory cells are quantified via fluorometer.

Recent Product Citations

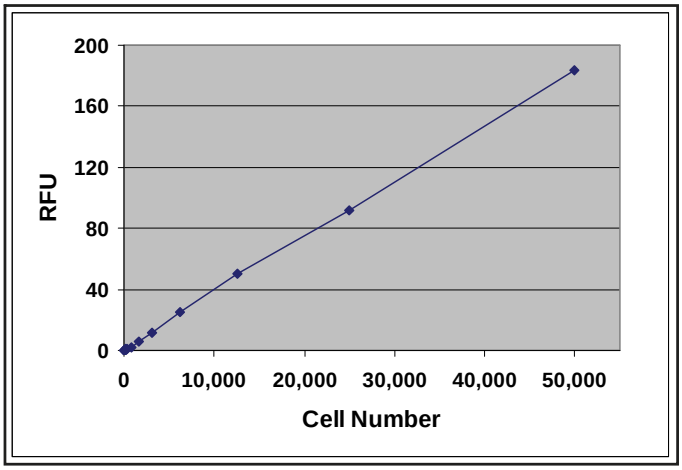
1. Fava, G. et al. (2008). Leptin enhances cholangiocarcinoma cell growth. *Cancer Res.* **68**:6752-6761. (CBA-212)
2. Xu, Z. et al. (2010). Role of pancreatic stellate cells in pancreatic cancer metastasis. *Am. J. Pathol.* **177**:2585-2596. (CBA-216)
3. Yang, H. and H.E. Grossniklaus (2010). Constitutive overexpression of pigment epithelium derived factor inhibition of ocular melanoma growth and metastasis. *Invest. Ophthalmol. Vis. Sci.* **51**:28-34. (CBA-216)



The Leukocyte Adhesion and Transmigration Cascade.



Assay Principle for the CytoSelect™ Leukocyte Transmigration Assay.



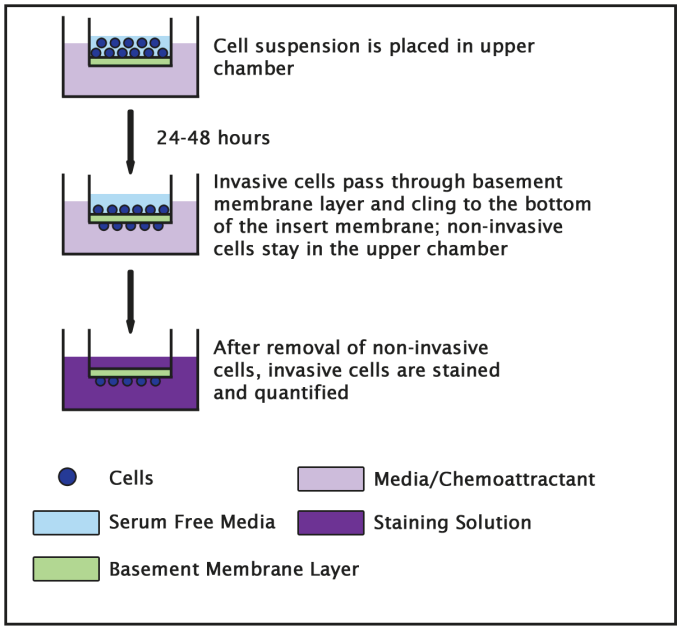
Quantitation of Human Monocytic THP-1. LeukoTracker™ labeled THP-1 cells were titrated in 1X PBS, then lysed with 2X lysis buffer. Fluorescence was quantified as described in the assay protocol.

Product Name	Pore Size	Detection	Size	Catalog Number
CytoSelect™ Leukocyte Transmigration Assay	3 µm	Fluorometric	24 Assays	CBA-212
CytoSelect™ Tumor Transendothelial Migration Assay	8 µm	Fluorometric	24 Assays	CBA-216

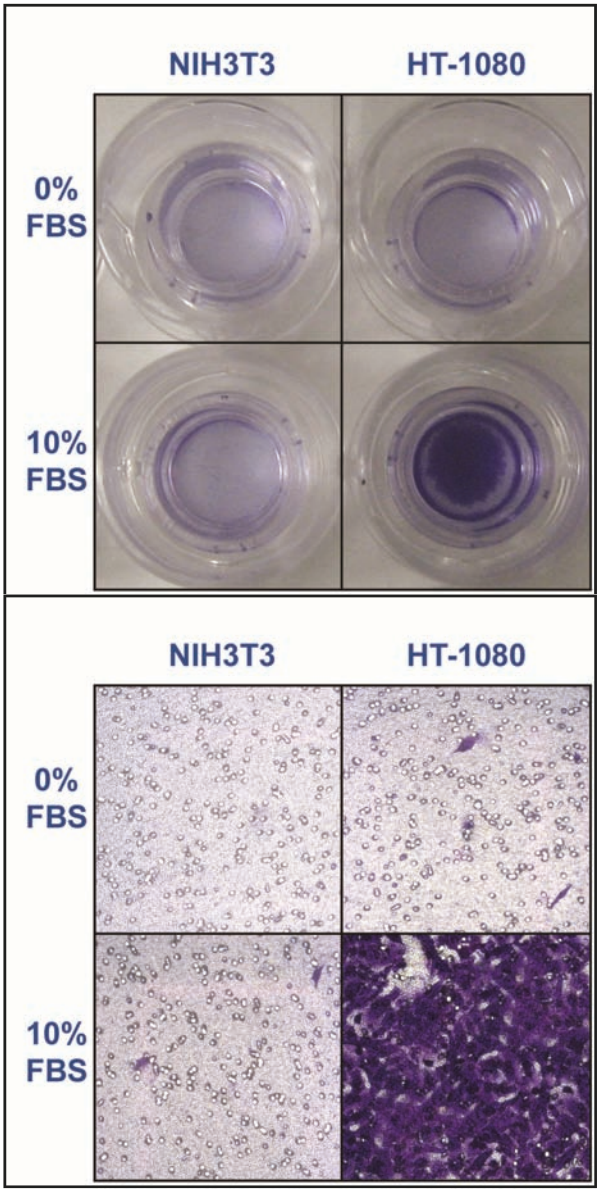
CytoSelect™ Cell Invasion Assays

Tumor cell invasion into surrounding normal tissue contributes to the morbidity of cancers. The CytoSelect™ Cell Invasion Assays use precoated inserts to assay invasive properties of tumor cells in 24-well or 96-well plates. The coated layer serves to distinguish invasive cells from non-invasive cells. Plates are precoated with either basement membrane matrix (from EHS mouse sarcoma cells), Collagen I or Laminin I.

- **Quantitative**: Measure results in a colorimetric or fluorescence plate reader
- **Flexible**: Uniform protein matrix layer of your choice of basement membrane (from mouse tumor cells), Collagen I, or Laminin I
- **Versatile**: Characterize both the invasive and migratory properties of your cells with a Cell Migration / Invasion Combo Kit (next page)



CytoSelect™ Cell Invasion Assay Principle.

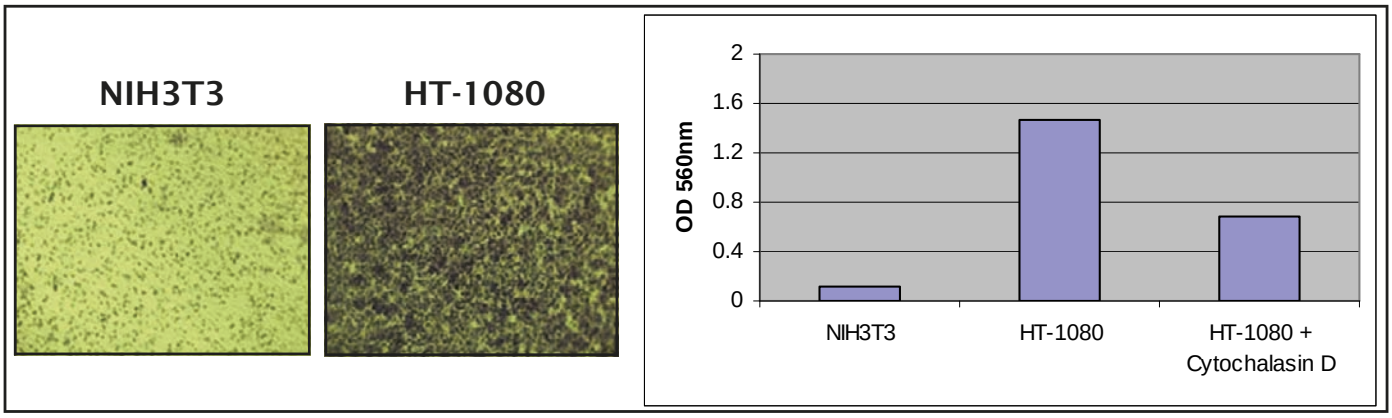


Human Fibrosarcoma HT-1080 Laminin I Cell Invasion. HT-1080 and NIH3T3 (negative control) were seeded at 200,000 cells/well and allowed to invade toward FBS for 24 hrs. Invasive cells on the membrane bottom were stained (top and center) and quantified at OD 560nm after extraction (data not shown).

Recent Product Citations

1. Zhang, L. et al. (2011). MicroRNA-1258 suppresses breast cancer brain metastasis by targeting heparanase. *Cancer Res.* **71**:645-654. (CBA-110)
2. Coon, B. et al. (2010). The epsin family of endocytic adaptors promotes fibrosarcoma migration and invasion. *J. Biol. Chem.* **285**:33073-33081. (CBA-110)
3. Hirata, H. et al. (2010). Role of secreted Frizzled-related protein3 in human renal cell carcinoma. *Cancer Res.* **70**:1896-1905. (CBA-110)
4. Ji, H. et al (2007). LKB1 modulates lung cancer differentiation and metastasis. *Nature* **48**:807-810. (CBA-110, CBA-111, CBA-112)
5. Eckstein, N. et al. (2009). Hyperactivation of the insulin-like growth factor receptor I signaling pathway is an essential event for cisplatin resistance of ovarian cancer cells. *Cancer Res.* **69**:2996-3003. (CBA-112)
6. Lam, K.K.W. et al. (2009). Glycodelin-A as a modulator of trophoblast invasion. *Hum. Reprod.* **24**:2093-2103. (CBA-112)
7. Neil, J.R. et al. (2008). Cox-2 inactivates Smad signaling and enhances EMT stimulated by TGFß through a PGE2-dependent mechanism. *Carcinogenesis* **29**:2227-2235. (CBA-112)
8. Thal, D.R. et al. (2008). Expression of coronin-3 (coronin-1C) in diffuse gliomas is related to malignancy. *J. Pathol.* **214**:415-424. (CBA-112)

CytoSelect™ Cell Invasion Assays, continued



Effects of Cytochalasin D on Invading Cells using the CytoSelect™ 24-well Cell Invasion Assay (CBA-110). HT-1080 and NIH3T3 cells (negative control) were seeded at 300,000 cells/well and allowed to invade toward 10% FBS for 24 hrs, in the presence or absence of 2 µM Cytochalasin D. Invasive cells, on the bottom of the invasion membrane, were stained (left) and then quantified at OD 560 nm after extraction using a standard plate reader (right).

Product Name	Detection	Size	Catalog Number
CytoSelect™ 24-Well Cell Invasion Assay, Basement Membrane	Colorimetric	12 Assays	CBA-110
	Fluorometric	12 Assays	CBA-111
CytoSelect™ 24-Well Cell Invasion Assay, Collagen I	Colorimetric	12 Assays	CBA-110-COL
	Fluorometric	12 Assays	CBA-111-COL
CytoSelect™ 24-Well Cell Invasion Assay, Laminin I	Colorimetric	12 Assays	CBA-110-LN
	Fluorometric	12 Assays	CBA-111-LN
CytoSelect™ 96-Well Cell Invasion Assay, Basement Membrane	Fluorometric	96 Assays	CBA-112
CytoSelect™ 96-Well Cell Invasion Assay, Collagen I	Fluorometric	96 Assays	CBA-112-COL
CytoSelect™ 96-Well Cell Invasion Assay, Laminin I	Fluorometric	96 Assays	CBA-112-LN

CytoSelect™ Cell Migration / Invasion Assay Combo Kits

Our CytoSelect™ Cell Migration / Invasion Assay Combo Kits allow you to characterize both the migratory and invasive properties of your cells. Each 24-well combo kit provides sufficient reagents to perform 12 migration plus 12 invasion assays, while the 96-well combo kit allows you to perform 96 migration plus 96 invasion assays. The invasion plate provided contains basement membrane-coated inserts.

Recent Product Citations

1. Shin, S.Y. et al. (2010). TNFalpha-exposed bone marrow-derived mesenchymal stem cells promote locomotion of MDA-MB-231 breast cancer cells through transcriptional activation of CXCR3 ligand chemokines. *J. Biol. Chem.* **285**:30731-30740. (CBA-100-C)
2. 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. (CBA-101-C)
3. Axlund, S.D. et al. (2010). HOXC8 inhibits androgen receptor signaling in human prostate cancer cells by inhibiting SRC-3 recruitement to direct androgen target genes. *Mol. Cancer Res*. **8**:1643-1655. (CBA-106-C)
4. Alfano, R.W. et al. (2009). Matrix metalloproteinase-activated anthrax lethal toxin inhibits endothelial invasion and neovasculature formation during in vitro morphogenesis. *Mol. Cancer Res.* **7**:452-461. (CBA-106-C)

Product Name	Pore Size	Detection	Size	Catalog Number
CytoSelect™ 24-Well Cell Migration / Invasion Combo Kit	8 µm	Colorimetric	2 x 12 Assays	CBA-100-C
		Fluorometric	2 x 12 Assays	CBA-101-C
CytoSelect™ 96-Well Cell Migration / Invasion Combo Kit	8 µm	Fluorometric	2 x 96 Assays	CBA-106-C

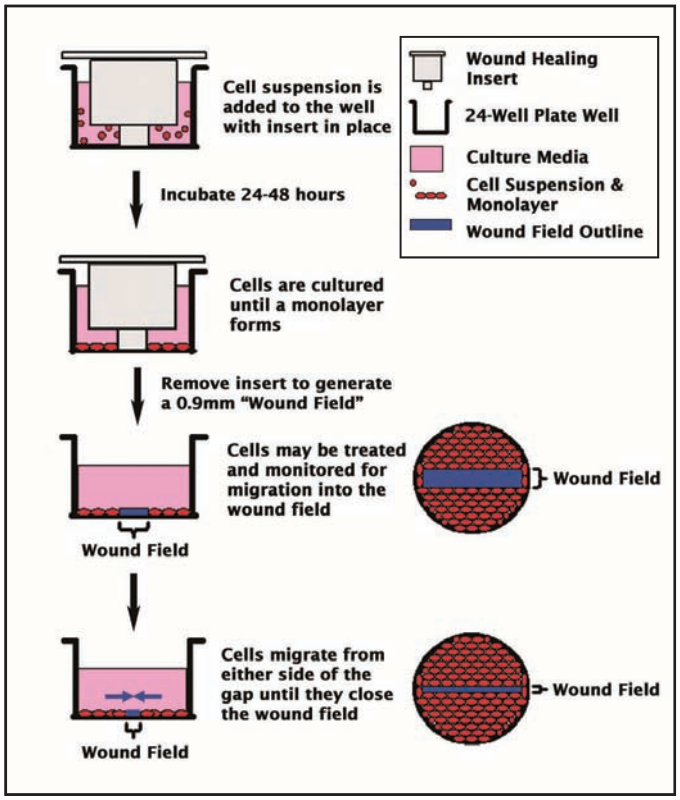
CytoSelect™ 24-Well Wound Healing / Cell Migration Assay

Wound healing assays are useful for studying tissue matrix remodeling, regulation of cytoskeletal structure, and cell proliferation and migration rates of different cells and culture conditions. Traditional wound healing assays are performed by making a scratch across a confluent cell monolayer to create an open gap, mimicking a "wound". Such scratch assays, however, lack a consistently defined wound area and result in high inter-sample variation.

Our CytoSelect™ 24-Well Wound Healing Assay provides a more consistent method to measure cell migration across a "wound field" gap *in vitro*. Proprietary treated inserts generate a consistently defined 0.9mm gap between the cells. Cells can then be treated and monitored for proliferation or migration across the wound field by imaging samples at fixed time points or time-lapse microscopy.

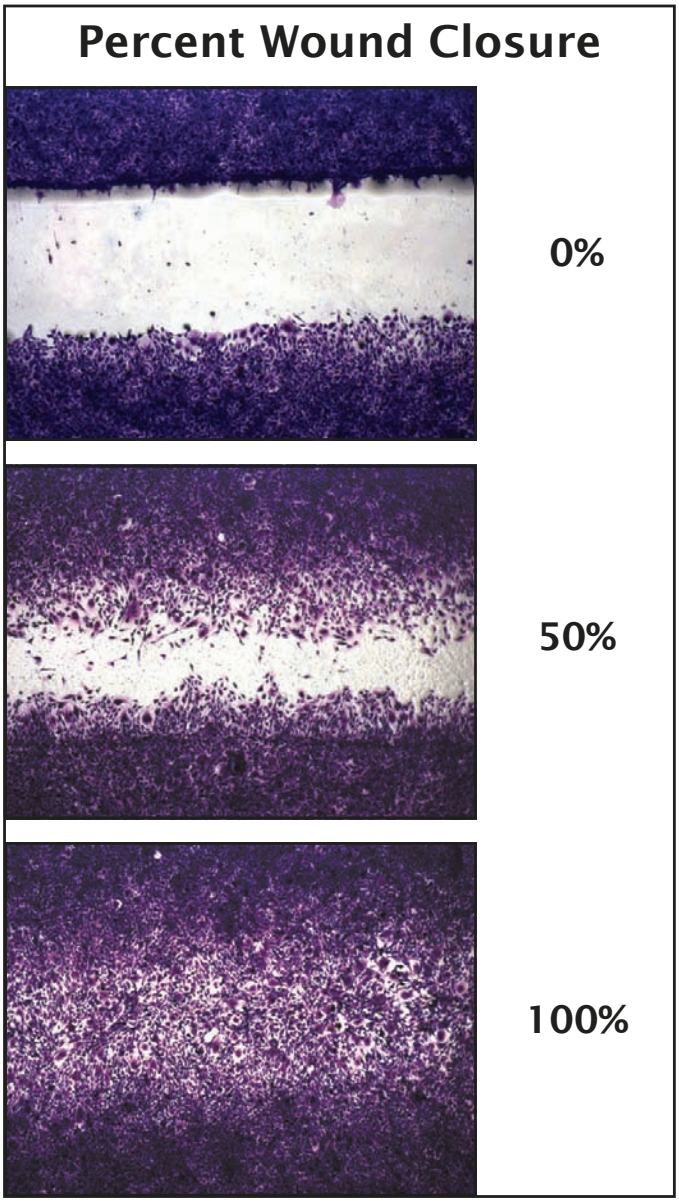
Recent Product Citation

Tao, Y. et al. (2011). Treatment of breast cancer cells with DNA demethylating agents leads to a release of Pol II stalling at genes with DNA-hypermethylated regions upstream of TSS. *Nucleic Acid Res*. 10.1093/nar/gkr611.



CytoSelect™ 24-well Wound Healing Assay Principle.

- **Highly Accurate**: More consistent results well-to-well compared to homemade scratch assays
- **Versatile**: Measure cell migration, cell proliferation, and wound closure
- **Inert Material**: No residues from inserts to impede cell migration or proliferation



Wound Closure of STO Cells. STO cells (mouse MEF) were cultured in the provided plate with inserts in place for 24 hours until a monolayer formed. Inserts were then removed to begin the assay. Cells were monitored at various time points and stained according to the assay protocol.

Product Name	Detection	Size	Catalog Number
CytoSelect™ 24-Well Wound Healing Assay	Microscopy	24 Assays	CBA-120
		5 x 24 Assays	CBA-120-5

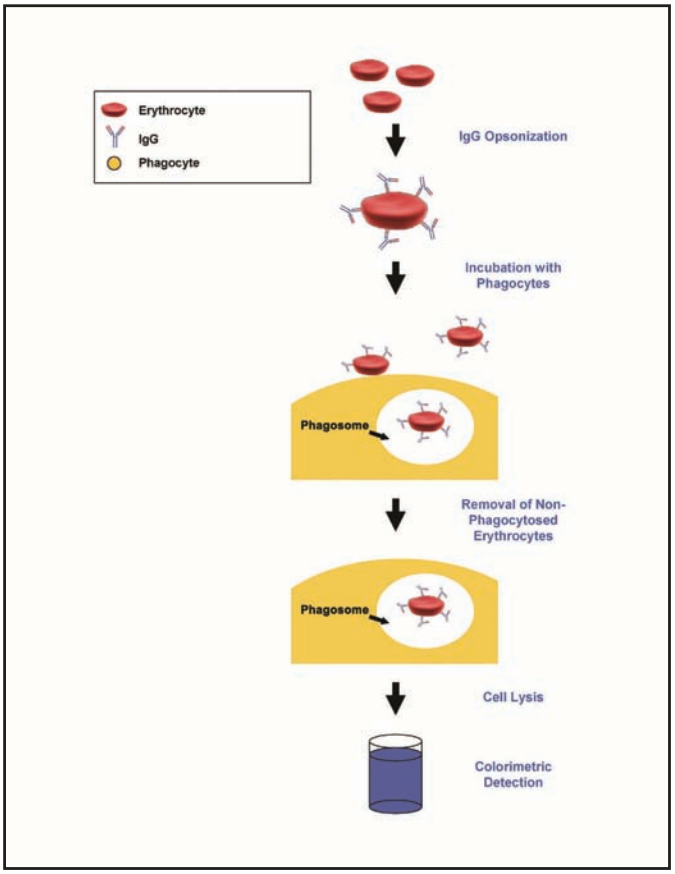
CytoSelect™ 96-Well Phagocytosis Assays

Phagocytosis may be assayed by measuring the engulfing of a cell "substrate" such as an erythrocyte (RBC) or Zymosan particle. Traditional phagocytosis assays involve manually counting the engulfed substrates under a microscope. This process is tedious and time-consuming, can be somewhat inaccurate, and is not amenable to high throughput.

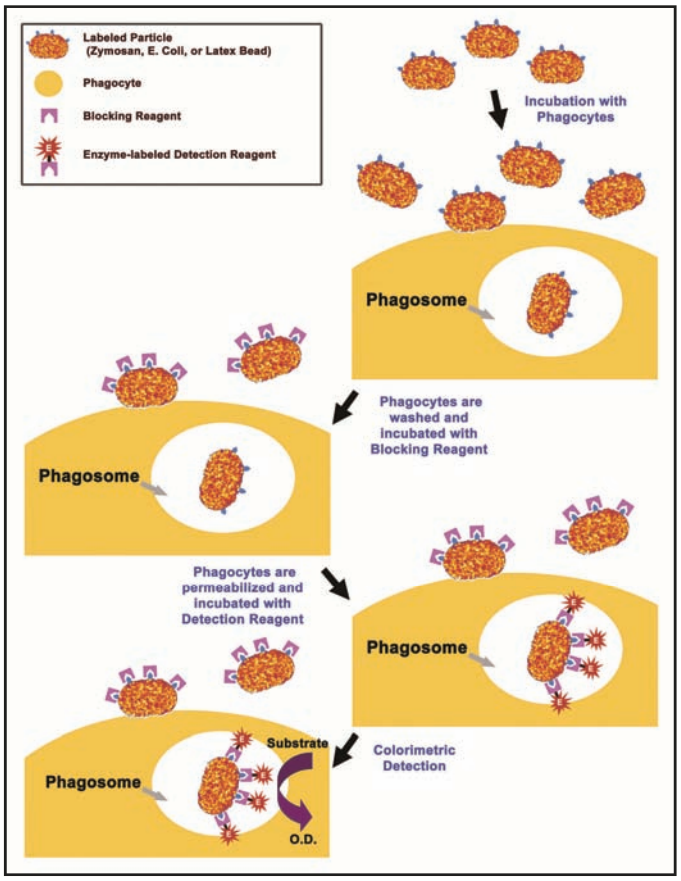
CytoSelect™ 96-Well Phagocytosis Assays are more accurate, high-throughput alternatives to the standard phagocytosis assay. The assays may be adapted for use in 48-well and 24-well plates if desired.

Recent Product Citations

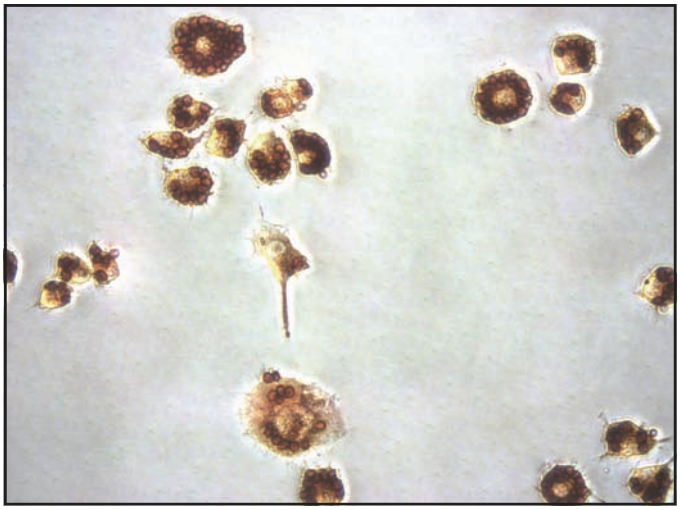
1. Lee, J.K. et al. (2010). Regulator of G-protein signaling-10 negatively regulates NF-kB in microglia and neuroprotects dopaminergic neurons in hemiparkinsonian rats. *J. Neurosci.* **31**:11879-11888. (CBA-220)
2. Winnicka, B. et al. (2010). CD13 is dispensible for normal hematopoiesis and myeloid cell functions in the mouse. *J. Leukoc. Biol.* **88(2)**:347-359. (CBA-220)



Assay Principle for the CytoSelect™ 96-Well Phagocytosis Assay (Red Blood Cell).



Assay Principle for the CytoSelect™ 96-Well Phagocytosis Assay (Zymosan).



Particle Engulfment with the CytoSelect™ 96-Well Phagocytosis Assay (Zymosan).

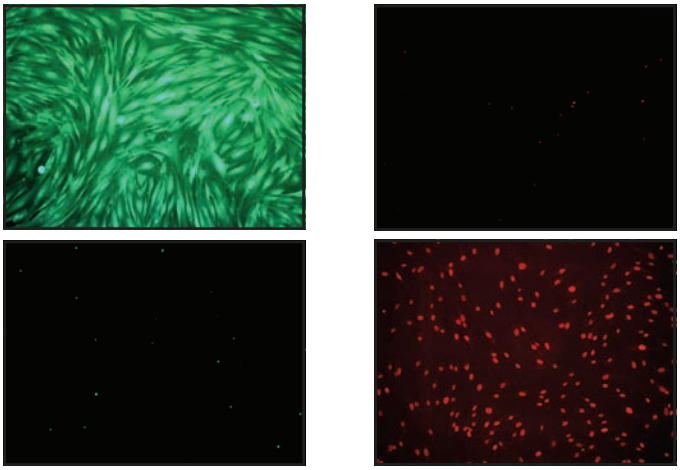
Product Name	Detection	Size	Catalog Number
CytoSelect™ 96-Well Phagocytosis Assay (Red Blood Cell)	Colorimetric	96 Assays	CBA-220
CytoSelect™ 96-Well Phagocytosis Assay (Zymosan)	Colorimetric	96 Assays	CBA-224

CytoSelect™ Cell Viability and Cytotoxicity Assay

Cell viability characteristics include cellular metabolic activity and cell membrane integrity. Our CytoSelect™ Cell Viability and Cytotoxicity Assay provides both a colorimetric and fluorometric format for monitoring cell viability via metabolic activity.

Live cells are detected with MTT (colorimetric detection) or Calcein AM (fluorometric); dead cells are detected with EthD-1 reagent (fluorometric). All 3 detection reagents are included, as well as Saponin, a cell death initiator. Cells may be treated with compounds or agents that affect cell viability. This kit is suitable for eukaryotic cells, not bacteria or yeast.

- **Versatile**: Detect live and dead cells by microscopy, colorimetric or fluorescence plate reader, or flow cytometry
- **Quantitative**: Measure live and dead cells on a fluorescence plate reader; live cells may also be quantified on a standard microplate reader



Viability of Human Foreskin Fibroblasts. BJ-TERT cells were seeded at 50,000 cells/well and allowed to culture for 24 hours. Cells were then treated with and without Saponin. All cells were then stained with Calcein AM and EthD-1. **Top**: Cells without Saponin treatment. **Bottom**: Cells with Saponin treatment. **Left**: Calcein AM staining. **Right**: EthD-1 staining.

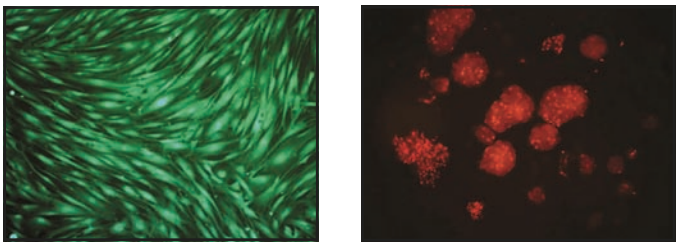
Product Name	Detection	Size	Catalog Number
CytoSelect™ Cell Viability and Cytotoxicity Assay Kit	Colorimetric / Fluorometric	1 plate*	CBA-240

*Each kit provides sufficient reagent quantities to perform 24 or 96 assays in a 24-well or 96-well plate respectively.

CytoSelect™ Anoikis Assays

This assay allows you to quantify and monitor anoikis in cells using a Poly-HEMA precoated plate. Live cells can be viewed under a microscope and quantified on a plate reader by MTT (colorimetric) or Calcein AM (fluorometric), both included with the kit. Dead cells are detected with EthD-1 reagent.

- **Versatile**: Detect live and dead cells by microscopy, fluorescence, or flow cytometry
- **Quantitative**: Measure live and dead cells on a fluorescence plate reader; live cells may also be quantified on a standard microplate reader



Anoikis of Human Fibroblast BJ-TERT Cells. 50,000 cells/well were seeded in a control plate (left) and a Poly-HEMA coated plate (right) and cultured for 24 hours. Cells on the control plate were stained with Calcein AM. Cells on the Poly-HEMA coated plate were stained with EthD-1.

Recent Product Citations

1. Sisto, M. et al. (2009). Fibulin-6 expression and anoikis in human salivary gland epithelial cells: implications in Sjogren's syndrome. *Int. Immunol.* **21**:303-311. (CBA-080)
2. Liu, H. et al (2008). Cysteine-rich protein 61 and connective tissue growth factor induce de-adhesion and anoikis of retinal pericytes. *Endocrinology* **149**:1666-1677. (CBA-080)

Product Name	Detection	Size	Catalog Number
CytoSelect™ 24-Well Anoikis Assay	Colorimetric / Fluorometric	24 Assays	CBA-080
CytoSelect™ 96-Well Anoikis Assay	Colorimetric / Fluorometric	96 Assays	CBA-081

Cellular Senescence Assays

Senescence Associated (SA) β-galactosidase is a common biochemical marker of cellular senescence. Cells expressing such markers have been identified *in vivo* in tissues. SA ß-Gal catalyzes hydrolysis of X-gal, which produces a blue color in senescent cells.

The results may be seen using our SA β-gal Staining Kit or by flow cytometry using our Quantitative Cellular Senescence Assay. For higher throughput, quantify cells on a fluorometric plate reader using our 96-Well Cellular Senescence Activity Assay.

Recent Product Citation

Malhotra, D. et al. (2010). Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. *Nucleic Acid Res*. 10.1093/nar/gkq212. (CBA-231)

Product Name	Detection	Size	Catalog Number
Cellular Senescence Assay Kit (SA β-gal Staining)	Light Microscopy	50 Assays	CBA-230
96-Well Cellular Senescence Assay (SA β-gal Activity)	Fluorometric Plate Reader	120 Assays	CBA-231
Quantitative Cellular Senescence Assay (SA β-gal)	Fluorescence Microscopy / Flow Cytometry	10 Assays	CBA-232

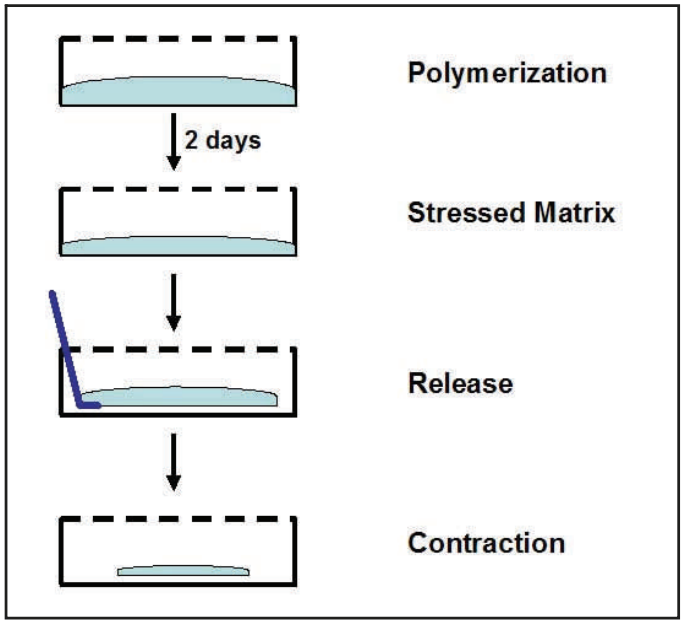
Cell Contraction Assay (Collagen-Based)

Wound healing is comprised of epithelialization, connective tissue deposition, and contraction. The contraction process is believed to be mediated by specialized fibroblasts (myofibroblasts). 3D collagen gels have been widely used in fibroblast contraction studies.

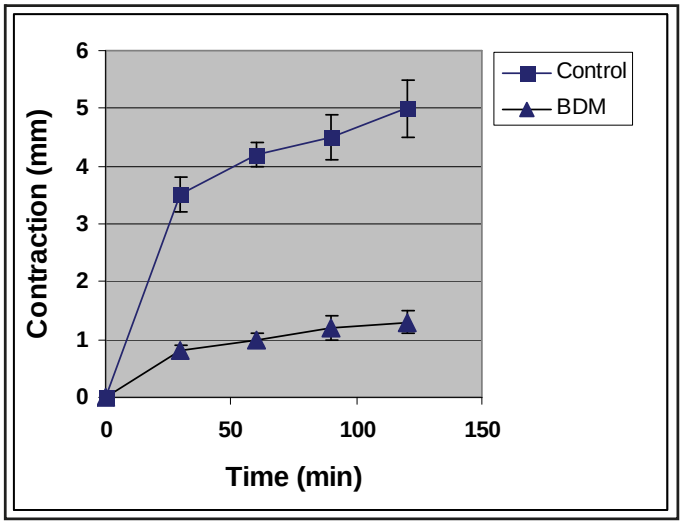
Our Cell Contraction Assay provides a simple system to assess cell contractivity and to screen for cell contraction mediators. The system uses a 3D collagen matrix to measure changes in the collagen gel size. An optional contraction inhibitor is provided.

Recent Product Citation

Schell, C. et al. (2010). 15-deoxy-delta12-14-prostaglandin-J2 induces hypertrophy and loss of contractility in human testicular peritubular cells: implications for human male fertility. *Endocrinology* **151**:12571268. (CBA-201)



Collagen-Based Cell Contraction Assay Principle.



Contraction Inhibition by BDM. 5 x 10^5 COS-7 cells in 0.5 mL collagen gel lattice were cultured for 2 days. Before initiation of contraction, cells were pretreated with 10mM BDM for 1 hour. The change in gel diameter (mm) was measured with a ruler at various times following release.

Product Name	Detection	Size	Catalog Number
Cell Contraction Assay	Light Microscopy	24 Assays	CBA-201

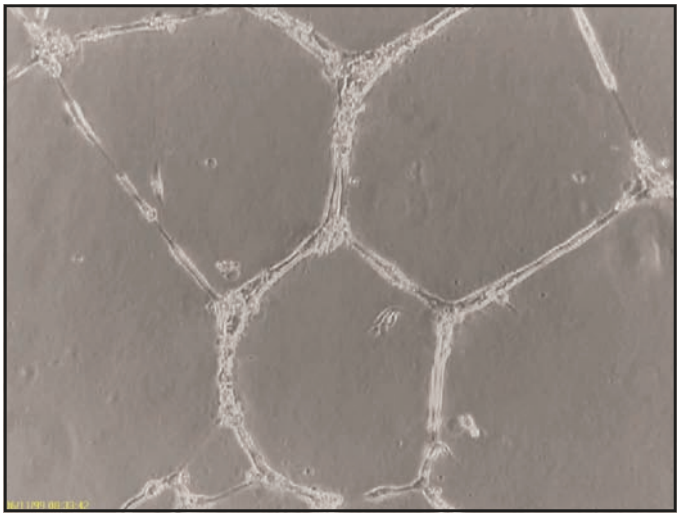
Endothelial Tube Formation (*In Vitro* Angiogenesis) Assay

For angiogenesis to occur, endothelial cells must escape their stable location and break through the basement membrane. Cells migrate toward an angiogenic stimulus that may be released from nearby tumor cells. These cells proliferate to form new blood vessels.

Our Endothelial Tube Formation Assay provides an easy, robust system to assess angiogenesis *in vitro*. The assay uses an ECM gel matrix derived from mouse sarcoma cells; this matrix very closely resembles an *in vivo* basement membrane environment.

Recent Product Citations

1. Kimura, T. et al. (2011). P2y5/LPA6 attenuates LPA1-mediated VE-cadherin translocation and cell-cell dissociation through G12/13 protein-Src-Rap1. *Cardiovasc. Res.* 10.1093/cvr/cvr154.
2. Hirata, H. et al. (2010). Role of secreted Frizzled-related protein3 in human renal cell carcinoma. *Cancer Res.* **70**:1896-1905.
3. Weskamp, G. et al. (2010). Pathological neovascularization is reduced by inactivation of ADAM17 in endothelial cells but not in pericytes. *Circ. Res.* **106**:932-940.
4. Alfano, R.W. et al. (2009). Matrix metalloproteinase-activated anthrax lethal toxin inhibits endothelial invasion and neovasculature formation during in vitro morphogenesis. *Mol. Cancer Res.* **7**:452-461.
5. Nogueras, S. et al. (2008). Coupling of endothelial injury and repair. An analysis using an in vivo experimental model. *Am. J. Physiol. Heart Circ. Physiol.* **294**:H708-H713.



HUVEC Tube Formation on ECM Gel. HUVEC cells from a standard tissue culture plate were incubated on an ECM gel. After several hours tube formation can be visualized under a light microscope.

Product Name	Detection	Size	Catalog Number
Endothelial Tube Formation Assay (*In Vitro* Angiogenesis)	Light Microscopy	50 Assays	CBA-200

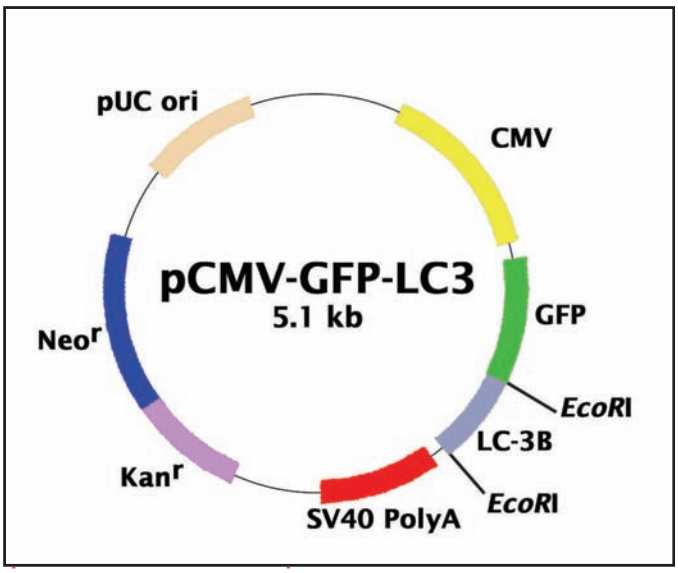
GFP-LC3 Expression Vectors

MAP LC3 is the most published autophagosome marker protein. LC3 associates to the inner and outer limiting membranes of the autophagosome. Two forms of LC3 are visible by immunoblot: LC3I found in the soluble fraction, and LC3II found in the membrane fraction. LC3II increases during autophagy.

Our GFP-LC3 expression vectors are available in three formats: mammalian, lentiviral, and retroviral expression. Each vector contains a GFP reporter gene, and a GFP control plasmid is included at no additional charge.

Recent Product Citation

Tu, S.P. et al. (2011). IFN-gamma inhibits gastric carcinogenesis by inducing epithelial cell autophagy and T-cell apoptosis. *Cancer Res.* **71**:4247-4259. (CBA-401)



Product Name	Size	Catalog Number
pCMV-GFP-LC3 Expression Vector	100 µL	CBA-401
pSMPUW-GFP-LC3 Lentiviral Expression Vector	10 µg	LTV-801
pMXs-GFP-LC3 Retroviral Expression Vector	10 µg	RTV-801

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