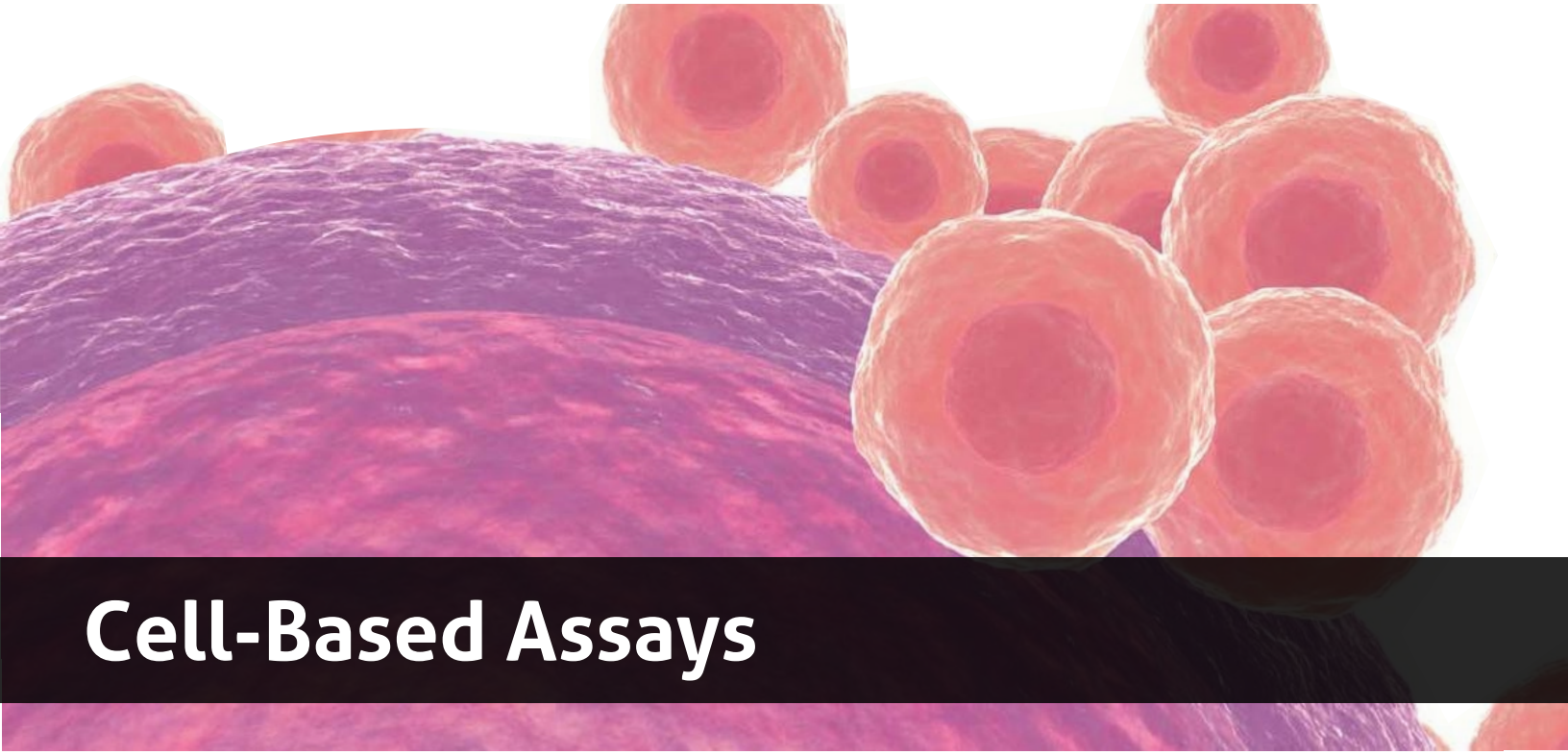
CELL BIOLABS, INC.
Creating Solutions for Life Science Research



Cell-Based Assays

- ☑ Cell Transformation / Colony Formation
- ☑ Cell Adhesion
- ☑ Cell Migration / Invasion
- ☑ Wound Healing
- ☑ Cell Vo-Culture
- ☑ Cell Health
- ☑ Cell Hypoxia
- ☑ Adipogenesis
- ☑ Phagocytosis
- ☑ Cell Contraction
- ☑ Angiogenesis
- ☑ Autophagy

Biogenuix®

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

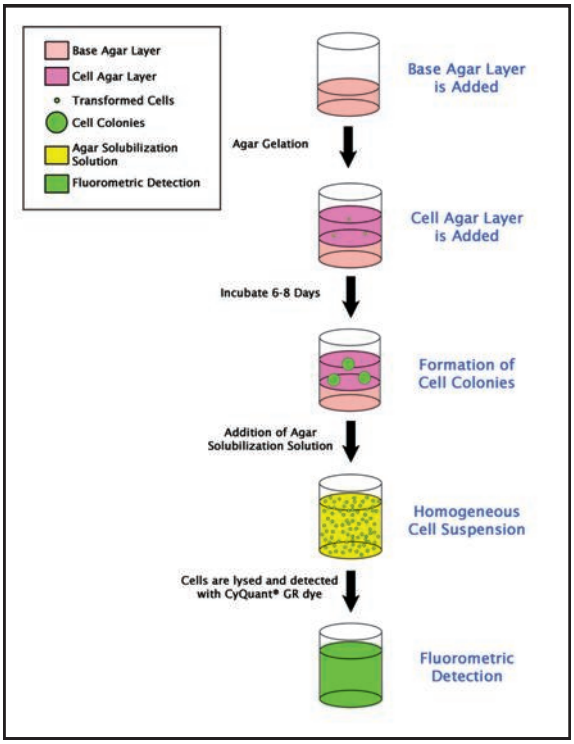
Our CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation) is suitable for measuring malignant transformation where no downstream analysis is required. Transformed cells cannot be recovered; however, no manual cell counting is required.

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. Tsukamoto, Y. et al. (2015). Expression of DDX27 contributes to colony forming ability of gastric cancer cells and correlates with poor prognosis in gastric cancer. *Am. J. Cancer Res.* **5**:2998.
2. Ercan, D. et al. (2015). EGFR mutations and resistance to irreversible pyrimidine based EGFR receptors. *Clin. Cancer Res.* **21**:3913-3923.
3. Mayr, C. et al. (2015). 3-Deazaneplanocin A may directly target putative cancer stem cells in biliary tract cancer. *Anticancer Res.* **35:**4697-4705.
4. Hua, G. et al. (2015). YAP induces high-grade serous carcinoma in fallopian tube secretory epithelial cells. *Oncogene* 10.1038/onc.2015.288.
5. Ukaji, T. et al. (2015). Inhibition of IGF-1 mediated cellular migration and invasion by migracin A in ovarian clear cell carcinoma cells. *PLoS One* **10**:e0137663.
6. Bon, H. et al. (2015). Salt-inducible Kinase 2 regulated mitotic progression and transcription in prostate cancer. *Mol. Cancer Res.* **13:**620-635.
7. Kim, T. et al. (2015). Role of MYC-regulated long noncoding RNAs in cell cycle regulation and tumorigenesis. *J. Nat. Cancer Inst.* **107**:dju505.

- **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



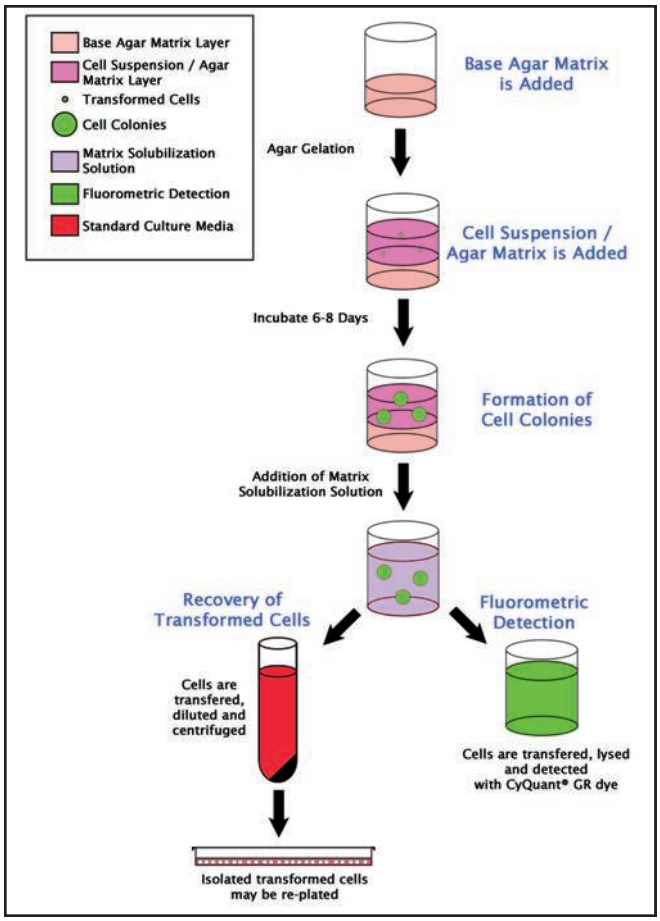
Cell Transformation Assay Principle.

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

*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.

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

Recent Product Citations

1. Mardin, B.R. et al. (2015). A cell-based model system links chromothripsis with hyperploidy. *Mol. Syst. Biol.* **11**:828. (CBA-135)
2. Monot, M. et al. (2015). Early steps of Jaagsiekte sheep retrovirus-mediated cell transformation involve the interaction between env and the RALBP1 cellular protein. *J. Virol.* **89**:8462-8473. (CBA-135)
3. Bon, H. et al. (2015). Salt-inducible Kinase 2 regulated mitotic progression and transcription in prostate cancer. *Mol. Cancer Res.* **13:**620-635. (CBA-135)
4. Fatemi, M. et al. (2014). Epigenetic silencing of CHD5, a novel tumor-suppressor gene, occurs in early colorectal cancer stages. *Cancer* **120**:172-180. (CBA-135)
5. Park, H. et al. (2014). Distinct roles of DKK1 and DKK2 in tumor angiogenesis. *Angiogenesis* **17**:221-234. (CBA-135)
6. Wang, X. et al. (2014). Commensal bacteria drive endogenous transformation and tumour stem cell marker expression through a bystander effect. *Gut* 10.1136/gutjnl-2014-307213. (CBA-135)
7. Bottero, V. et al. (2013). Kaposi's Sarcoma-associated Herpesvirus-positive primary effusion lymphoma tumor formation in NOD/SCID mice is inhibited by neomycin and neamine blocking angiogenin-s nuclear translocation. *J. Virol*. **87**:11806-11820. (CBA-135)
8. Singh, R. et al. (2013). Increasing the complexity of chromatin: functionally distinct roles for replication-dependent histone H2A isoforms in cell proliferation and carcinogenesis. *Nucleic Acids Res.* 10.1093/nar/gkt736. (CBA-135)
9. Shukla, A. et al. (2013). Extracellular signal-regulated kinase 5: a potential therapeutic target for malignant mesotheliomas. *Clin. Cancer Res.* **19**:2071-2083. (CBA-135)
10. Niccoli, S. et al. (2012). The Asian-American E6 variant protein of human papillomavirus 16 alone is sufficient to promote immortalization, transformation, and migration of primary human foreskin keratinocytes. *J. Virol.* **86**:12384-12396. (CBA-135)
11. Hong, S.W. et al. (2012). Ring finger protein 149 is an E3 ubiquitin ligase active on wild-type v-Raf murine sarcoma viral oncogene homolog B1 (BRAF). *J. Biol. Chem.* **287**:24017-24025. (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.

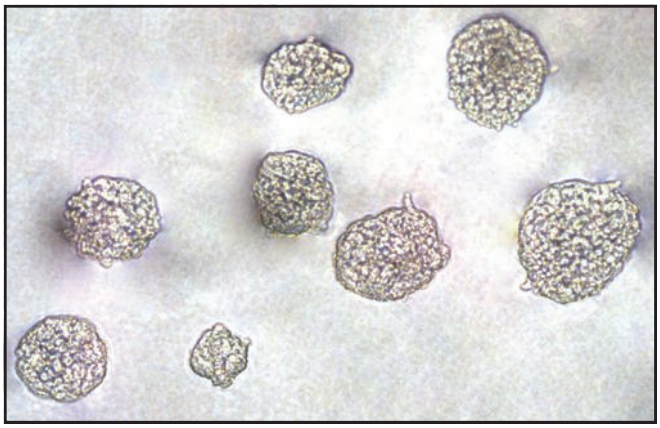
CytoSelect™ 96-Well *In Vitro* Tumor Sensitivity Assay

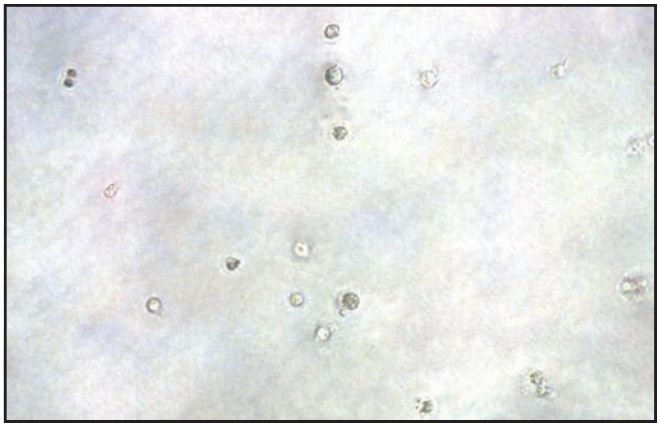
The CytoSelect™ *In Vitro* Tumor Sensitivity Assay provides a stringent, anchorage-independent model for chemosensitivity testing and possible anticancer drug screening. The assay uses a soft agar matrix to promote the colony formation of neoplastic cells in about a week. Cells are quantified using a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

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

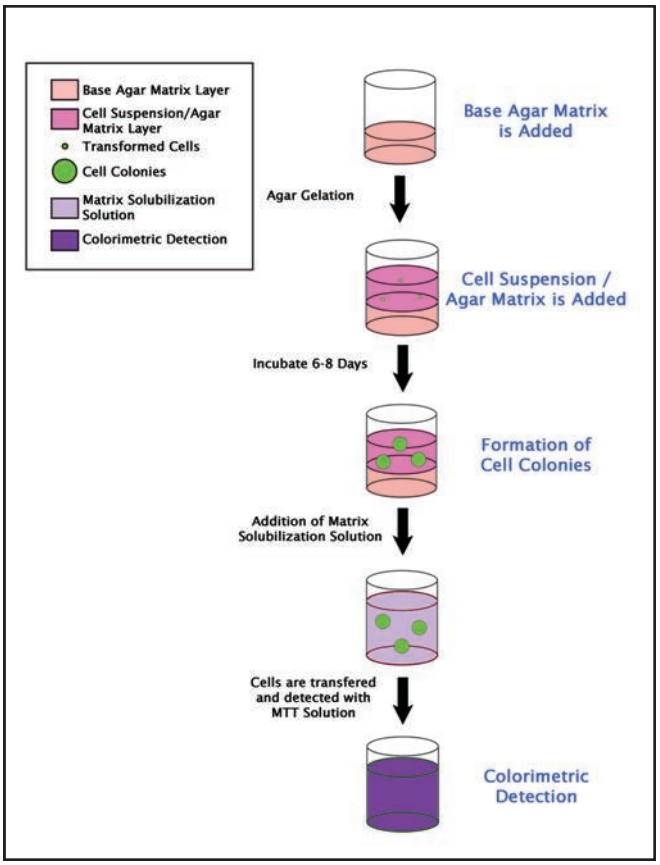
Recent Product Citations

1. Meador, C.B. et al. (2015). Optimizing the sequence of anti-EGFR-targeted therapy in EGFR-mutant lung cancer. *Mol. Cancer Ther.* **14**:542-552.
2. Aki, M.R. et al. (2015). Araguspongine C induces autophagic death in breast cancer cells through suppression of c-Met and HER2 receptor tyrosine kinase signaling. *Mar Drugs* **13**:288-311.
3. Peng, Y.T. et al. (2015). Upregulation of cyclin-dependent kinase inhibitors CDKN1B and CDKN1C in hepatocellular carcinoma-derived cells via goniothalamin-mediated protein stabilization and epigenetic modifications. *Toxicol. Rep*. 10.1016/j.toxrep.2015.
4. Suman, S. et al. (2014). The pro-apoptotic role of autophagy in breast cancer. *Bri. J. Cancer* **111**:309-317.
5. Bard-Chapeau, E. et al. (2013). EVI1 oncoprotein interacts with a large and complex network of proteins and integrates signals through protein phosphorylation. *PNAS* **110**:E2885-E2894.
6. Takezawa, K. et al. (2012). HER2 amplification: a potential mechanism of acquired resistance to EGFR inhibition in EGFR-mutant lung cancers that lack the second-site EGFRT790M mutation. *Cancer Discovery* **2**:922-933.





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.

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

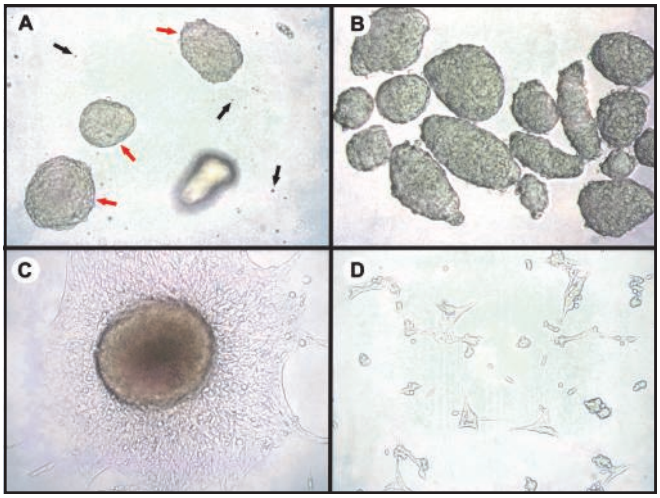
CytoSelect™ Clonogenic Tumor Cell Isolation Kit

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.

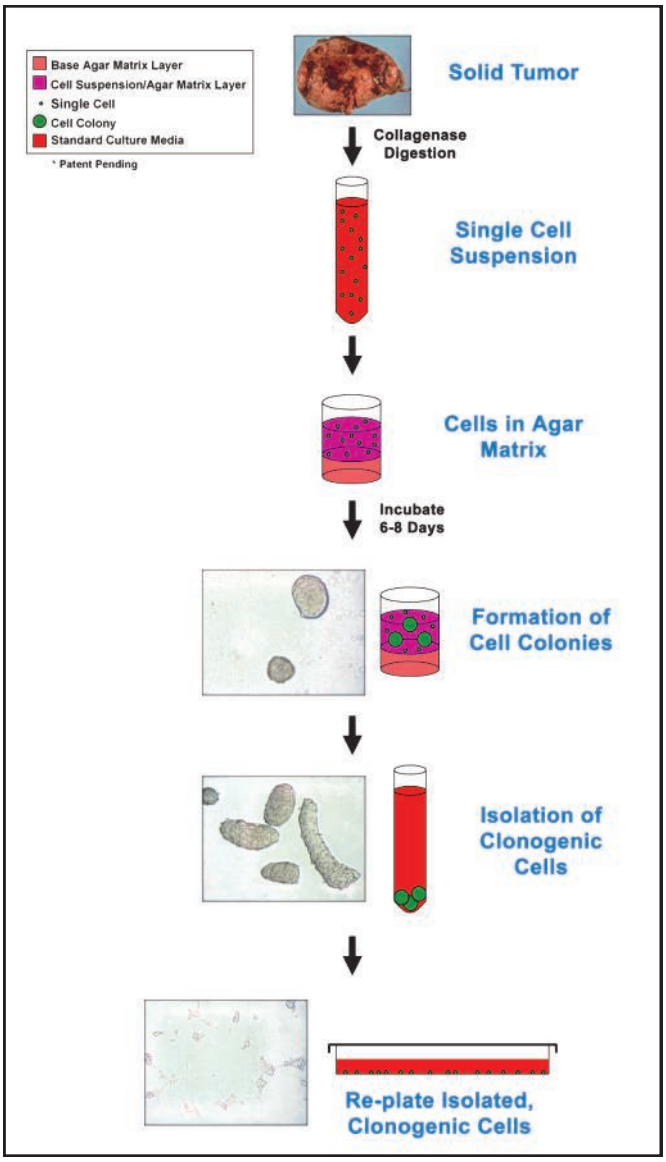
The CytoSelect™ Clonogenic Tumor Cell Isolation Kit uses a proprietary semisolid agar medium to facilitate colony formation by cells from solid tumors.

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

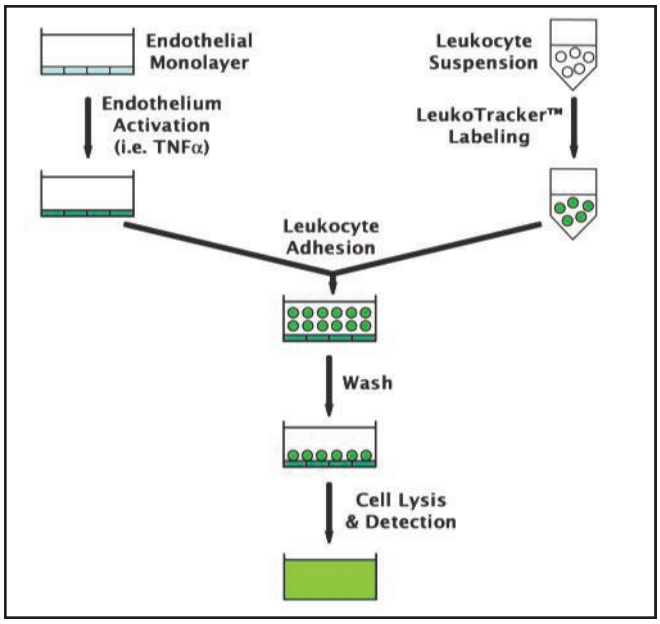
Cell Adhesion Assays

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™ 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

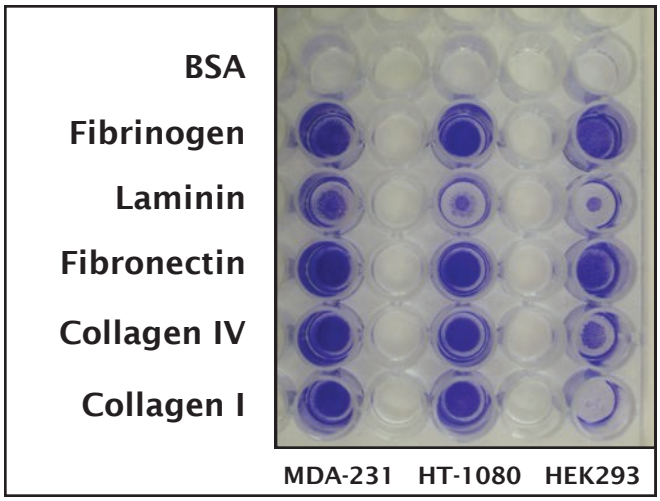
1. Huang, M. et al. (2015). Niclosamide inhibits the inflammatory and angiogenic activation of human umbilical vein endothelial cells. *Inflamm. Res*. **64**:1023-1032. (CBA-210)
2. Shah, D. et al. (2015). C1q deficiency promotes pulmonary vascular inflammation and enhances the susceptibility of the lung endothelium to injury. *J. Biol. Chem*. 10.1074/jbc.M115.690784. (CBA-210)
3. Campos-Estrada, C. et al. (2015). Simvastatin and benznidazole-mediated prevention of Trypanosoma cruzi-induced endothelial activation: role of 15-epi-lipoxin AA4 in the action of simvastatin. *PLoS Negl Trop. Dis*. **9**:e0003770. (CBA-210)
4. Ibrahim, A.S. et al. (2015). A lipidomic screen of hyperglycemia-treated HRECs links 12/15-lipoxygenase to microvascular dysfunction during diabetic retinopathy via NADPH oxidase. *J. Lipid Res.* **56**:599-611. (CBA-210)
5. Cao, Q. et al. (2014). Inhibiting DNA methylation by 5-aza-2'-deoxycytidine ameliorates atherosclerosis through suppressing macrophage inflammation. *Endocrinology* **155**:4925-4938. (CBA-210)
6. Kapitsinou, P.P. et al. (2014). Endothelial HIF-2 mediates protection and recovery from ischemic kidney injury. *J. Clin. Invest.* **124**:2396. (CBA-210)
7. Wu, X.Y. et al. (2014). Regulation of microRNA-515 in endothelial inflammation by targeting nuclear factor (NF)-kappaB p65. *J. Cell Biochem*. **115**:1928-1936. (CBA-210)
8. Ghoshal, P. et al. (2014). Glycosylation inhibitors efficiently inhibit P-selectin-mediated cell adhesion to endothelial cells. *PLoS One* **9**:e99363. (CBA-210)
9. Jassam, S.A. et al. (2015). TNF-alpha enhancement of CD62E mediates adhesion of non-small cell lung cancer cells to brain endothelium via CD15 in lung-brain metastasis. *Neuro Oncol*. 10.1093/neuonc/nov248. (CBA-215)
10. Finetti, F. et al. (2015). mPGES-1 in prostate cancer controls stemness and amplifies EGFR-driven oncogenicity. *Endocr. Relat. Cancer* 10.1530/ERC-15-0277. (CBA-215)
11. Yang, M. et al. (2014). IgG expression in trophoblasts derived from placenta and gestational trophoblastic disease and its role in regulating invasion. *Immunol. Res.* **60**:91-104. (CBA-215)
12. Sasahira, T. et al. (2014). Transport and Golgi organisation protein 1 is a novel tumour porgrdesssive factor in oral squamous cell carcinoma. *Eur. J. Cancer* **50**:2142-2151. (CBA-215)

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™ ECM Cell Adhesion Assays

CytoSelect™ ECM Cell Adhesion Assays provide a quantitative method to measure cell adhesion. The 48 -well plate is precoated with your choice of substrate. Adherent cells attach, while non-adherent cells are washed away. Adherent cells can be quantified on a colorimetric or fluorometric plate reader.

- **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



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

Recent Product Citations

1. Jiang, F. et al. (2015). CYP3A5 functions as a tumor suppressor in hepatocellular carcinoma by regulating mTORC2/Akt signaling. *Cancer Res*. **75**:1470-1481. (CBA-050)
2. Chen, L. et al. (2015). Both mTORC1 and mTORC2 are involved in the regulation of cell adhesion. *Oncotarget*. **6**:7136-7150. (CBA-050 and CBA-056)
3. Aghajanin, H. et al. (2014). Semaphorin 3d and semaphorin 3e direct endothelial motility through distinct molecular signaling pathways. *J. Biol. Chem*. **289**:17971-17979. (CBA-052)
4. Lee, J. et al. (2013). Selective inhibition of prostaglandin E2 receptors EP2 and EP4 inhibits adhesion of human endometriotic epithelial and stromal cells through suppression of integrin-mediated mechanisms. *Biol. Reprod*. **88**:77. (CBA-057)
5. Montealegre, M.C. et al. (2015). The *Enterococcus faecalis* EbpA pilus protein: attenuation of expression, biofilm formation, and adherence to fibrinogen start with the rare initiation codon ATT. *MBio.* **6**:e00467-15. (CBA-058)
6. 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)
7. Nowakowska, M. et al. (2014). Diverse effect of WWOX overexpression in HT29 and SW480 colon cancer cell lines. *Tumor Biol.* **35**:9291-9301. (CBA-070)
8. Singla, A.K. et al. (2015). Characterization of a murine model of metastatic human non-small cell lung cancer and effect of CXCR4 inhibition on the growth of metastases. *Oncoscience* **2**:263-271. (CBA-070)
9. Jain, M. et al. (2014). ZHF367 inhibits cancer progression and is targeted by miR-195. *PLoS One* **9**:e101423. (CBA-070)
10. Tanoury, Z. et al. (2014). Genes involved in cell adhesion and signaling: a new repertoire of retinoic acid receptor target genes in mouse embryonic fibroblasts. *J. Cell Sci.* **127**:521-533. (CBA-070)
11. Aohra, F.T. et al. (2015). Functional behavior and gene expression of magnetic nanoparticle-loaded primary endothelial cells for targeting vascular stents. *Nanomedicine (Lond.)* **10**:1391-1406. (CBA-071)

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
		5 x 48 Assays	CBA-070-5
	Fluorometric	48 Assays	CBA-071
		5 x 48 Assays	CBA-071-5
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

Cell Migration & Invasion Assays

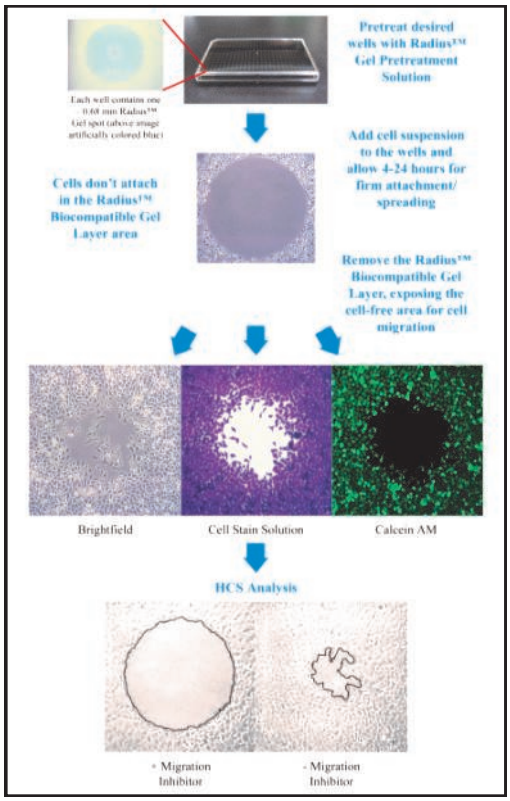
Cell migration and invasion are highly integrated, multi-step processes and play important roles in the progression of various diseases including cancer, atherosclerosis and arthritis.

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.

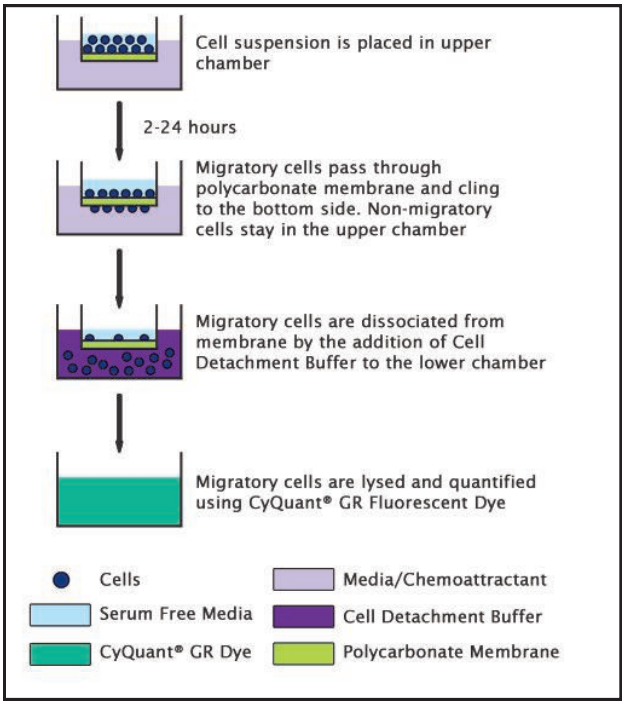
Cell invasion assays are provided in the Boyden Chamber format.

Cell Migration Format Selection Guide

	2D Gap Closure Assays (p. 13)	Boyden Chamber Assays (p. 14-19)
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 pore size based on cell size



Assay Principle for the Radius™ 2D Gap Closure Assays.



Example of Boyden Chamber Assay Principle.

Radius™ Cell Migration Assays (2D Gap Closure)

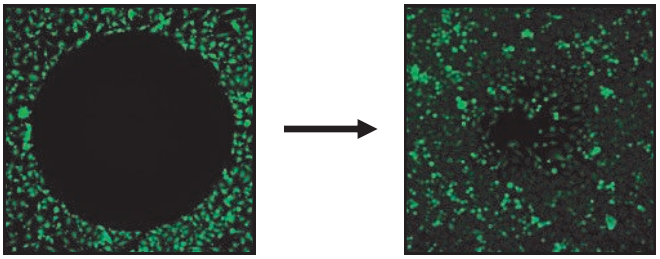
Radius™ Cell Migration Assays provide a unique alternative to the traditional Boyden Chamber migration assay. Radius assays allow you to measure cell migration at endpoint or in real time, and are ideal for time course migration studies.

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



Example Results using 2D Gap Closure Assay.

Recent Product Citations

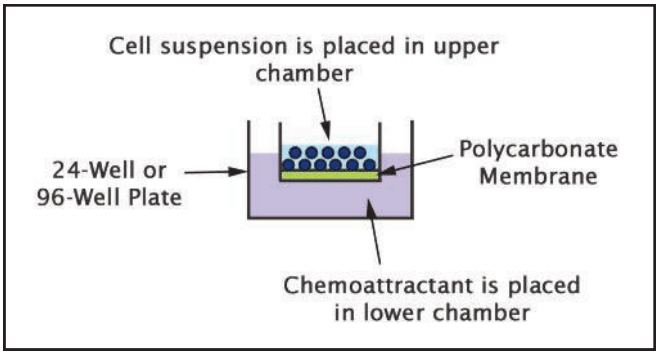
1. Sanna, V. et al. (2015). Nanoencapsulation of natural triterpenoid celastrol for prostate cancer treatment. *Int. J. Nanomedicine* **10**:6835-6846. (CBA-125)
2. Nishikawa, M. et al. (2015). Enhanced sensitivity to sunitinib by inhibition of Akt1 expression in human castration-resistant prostate cancer PC3 cells both in vitro and in vivo. *Urology* **85**:1215-e1. (CBA-125)
3. Pu, J. et al. (2015). BORC, a multisubunit complex that regulates lysosome positioning. *Dev. Cell* **33**:176-188. (CBA-125)
4. Ge, C. et al. (2015). Role of Runx2 phosphorylation in prostate cancer and association with metastatic disease. *Oncogene* 10.1038/onc.2015.91. (CBA-125)
5. Kim, E.K. et al. (2015). First evidence that ecklonia cava-derived dieckol attenuates MCF-7 human breast carcinoma cell migration. *Mar Drugs* **13**:1785-1797. (CBA-125)
6. Camacho, M. et al. (2015). Prostacyclin-synthase expression in head and neck carcinoma patients and its prognostic value in the response to radiotherapy. *J. Pathol.* **235**:125-135. (CBA-126)
7. Woodard, G.E. et al. (2015). Characterization of discrete subpopulations of progenitor cells in traumatic human extremity wounds. *PLoS One* **9**:e114318. (CBA-126)
8. Felthaus, O. et al. (2014). Migration of human dental follicle cells in vitro. *J. Periodontal Res*. **49**:205-212. (CBA-126)
9. Wong, B. et al. (2013). Adrenomedullin enhances invasion of human extravillous cytotrophoblast-derived cell lines by regulation of urokinase plasminogen activator expression and S-nitrosylation. *Biol. Reprod.* **88**:34. (CBA-126)
10. Ichikawa, A. et al. (2013). CXCL10-CXCR3 enhances the development of neutrophil-mediated fulminant lung injury of viral and nonviral origin. *Am. J. Respir. Crit. Care Med.* **187**:65-77. (CBA-126)

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.

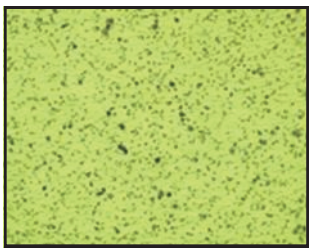
Boyden Chamber Assay Selection Guide

Assay	Definition	Cell Types	Pore Size	Insert Coating	Assay Formats
Chemotaxis (p. 15)	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. 16)	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. 17)	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. 18-19)	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

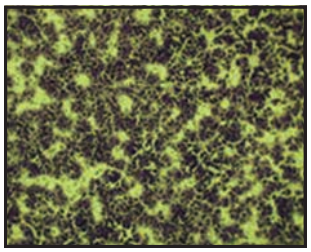
CytoSelect™ Cell Migration Assays—Chemotaxis

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



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. Ran, X. et al. (2015). A quantitative proteomics study on olfactomedin 4 in the development of gastric cancer. *In. J. Oncol.* **47**:1932-1944. (CBA-100)
2. Koenig, J. et al. (2015). Placental mesenchymal stromal cells derived from blood vessels or avascular tissues: what is the better choice to support endothelial cell function? *Stem Cells Dev.* **24**:115-131. (CBA-100)
3. Banerjee, D. et al. (2015). Notch suppresses angiogenesis and progression on hepatic metastases. *Cancer Res.* **75**:1592-1602. (CBA-101)
4. Deng, B. and Feng, Y. (2015). TIPE2 mediates the suppressive effects of Shikonin on MMP13 in osteosarcoma cells. *Cell Physiol. Biochem.* **37**:2434-2443. (CBA-102)
5. Sloniecka, M. et al. (2015). Substance P enhances keratocyte migration and neutrophil recruitment through interleukin-8. *Mol. Pharmacol.* 10.1124/mol.115.101014. (CBA-103)
6. Kitano, K. et al. (2014). Rho-kinase activation in leukocytes plays a pivotal role in myocardial ischemia/reperfusion injury. *PLoS One* **9**:e92242. (CBA-104)
7. Hargarten, J.C. et al. (2015). *Candida albicans* quorum sensing molecules stimulate mouse macrophage migration. *Infect. Immun.* **83**:3857-3864. (CBA-105)
8. Kondo, Y. et al. (2015). Differential expression of CX3CL1 in hepatitis B virus-replicating hepatoma cells can affect the migration activity of CX3CR1+ immune cells. *J. Virol*. **89**:7016-7027. (CBA-105)
9. Adam, M.G.. et al. (2015). SIAH ubiquitin ligases regulate breast cancer cell migration and invasion independent of the oxygen status. *Cell Cycle* **14**:3734-3747. (CBA-106)
10. Hammer, K. et al. (2015). Engineered adenoviruses combine enhanced oncolysis with improved virus production by mesenchymal stromal carrier cells. *Int. J. Cancer* 10.1002/ijc.29442. (CBA-107)

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

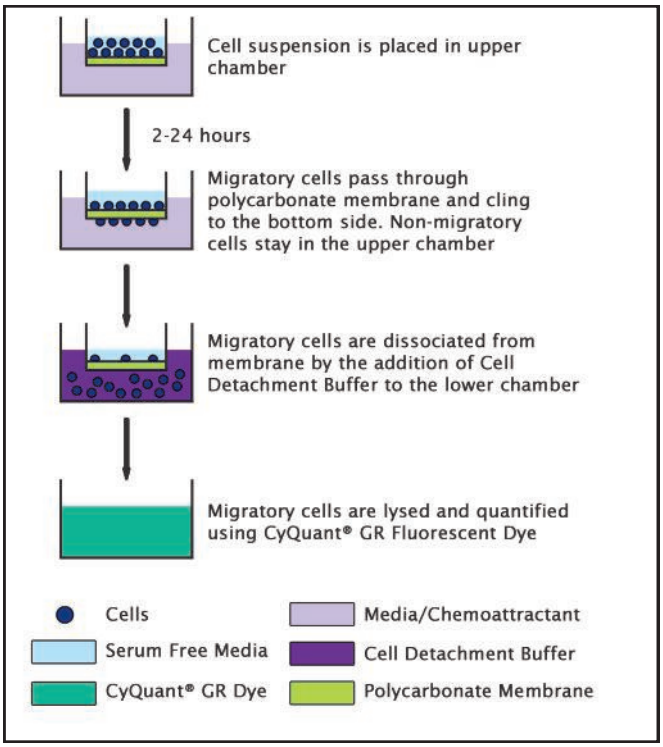
CytoSelect™ Cell Migration Assays—Haptotaxis

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.

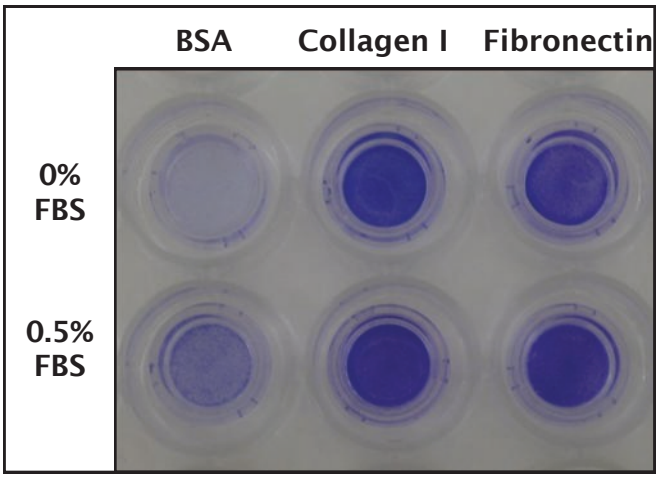
The undersides of the inserts are coated with either Collagen or Fibronectin. The 8 µm pore size in the membrane inserts is ideal for epithelial cells, endothelial cells, fibroblasts, and other cells of similar size. The membrane serves as a barrier that allows discrimination of migratory cells from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

- **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™ Chemotaxis Assays (previous page) or the CytoSelect™ 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.

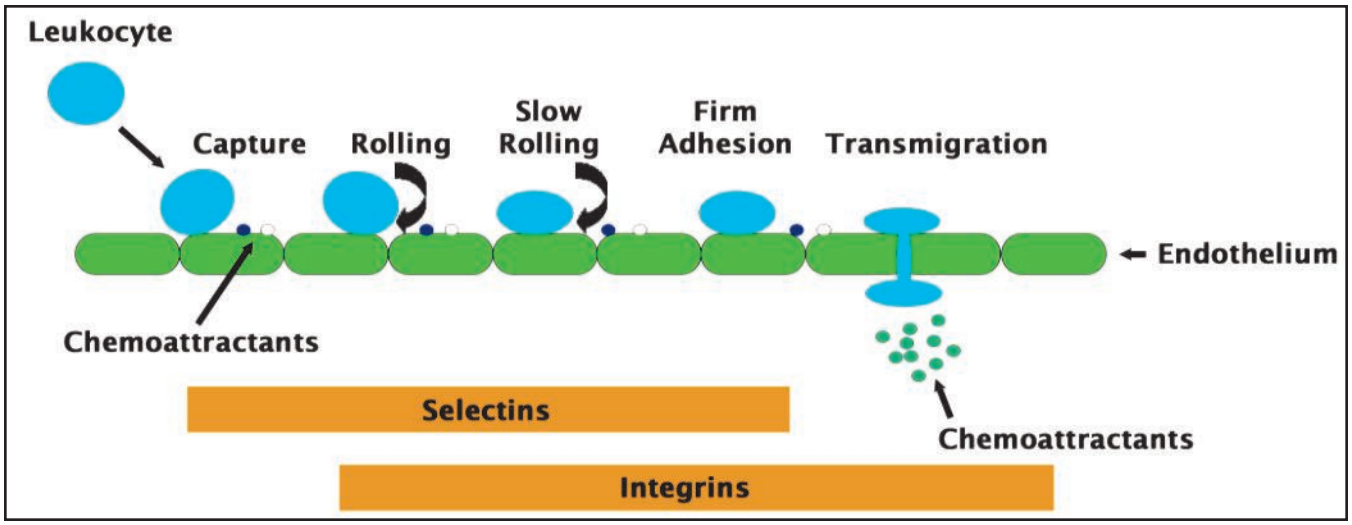
Recent Product Citations

1. Herrera, I. et al. (2013). Matrix metalloproteinase (MMP)-1 induces lung alveolar epithelial cell migration and proliferation, protects from apoptosis, and represses mitochondrial oxygen consumption. *J. Biol. Chem.* **288**:25964-25975. (CBA-100-COL)
2. Niccoli, S. et al. (2012). The Asian-American E6 variant protein of human papillomavirus 16 alone is sufficient to promote immortalization, transformation, and migration of primary human foreskin keratinocytes. *J. Virol.* **86**:12384-12396. (CBA-110-COL)
3. 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)
4. Singh, D.R. et al. (2015). EphA2 unliganded dimers suppress EphA2 pro-tumorigenic signaling. *J. Biol. Chem*. 10.1074/jbc.M115.676866. (CBA-101-COL)

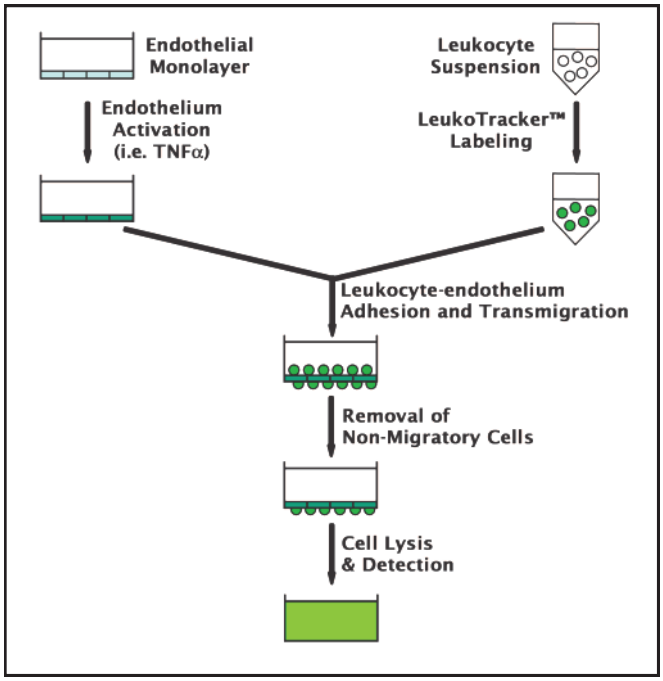
Product Name	Detection	Size	Catalog Number
CytoSelect™ 24-Well Cell Haptotaxis Assay, Collagen I-coated	Colorimetric	12 Assays	CBA-100-COL
	Fluorometric	12 Assays	CBA-101-COL
CytoSelect™ 24-Well Cell Haptotaxis Assay, Fibronectin-coated	Colorimetric	12 Assays	CBA-100-FN
	Fluorometric	12 Assays	CBA-101-FN

CytoSelect™ Cell Migration Assays—Transmigration

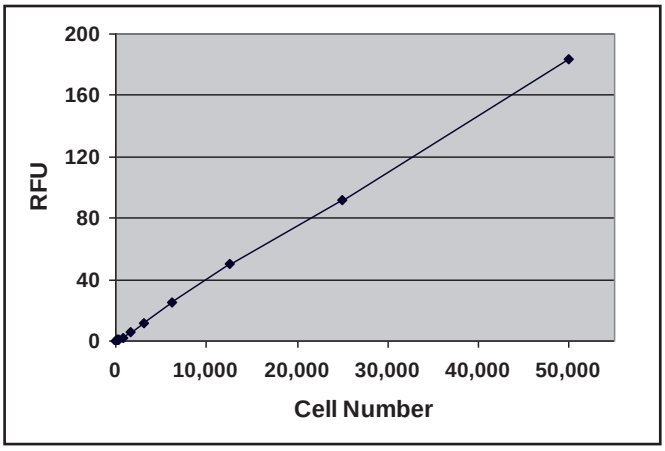
Cancer cell transmigration, particularly extravasation, is an important step in cancer metastasis. The CytoSelect™ Cell Transmigration Assays provide a robust system for the quantitation of transmigrations and interactions between endothelium and cancer cells. Migratory cells are quantified via fluorometer.



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.

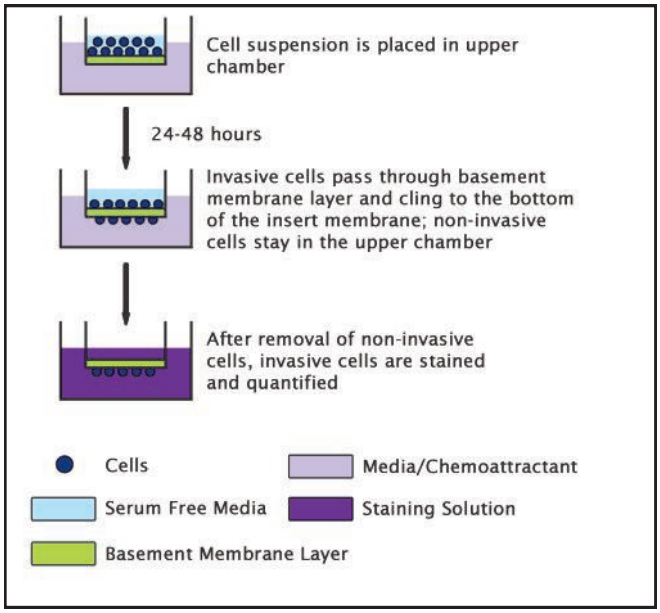
Recent Product Citations

1. Giunzioni, I. et al. (2015). Cigarette smoke condensate affects monocyte interaction with endothelium. *Atherosclerosis* **234**:383-390. (CBA-212)
2. Park, G.B. et al. (2014). The Epstein-Barr Virus causes epithelial -mesenchymal transition in human corneal epithelial cells via Syk/Src and Akt/ERK signaling pathways. *Invest. Ophthalmol. Vis. Sci.* **55**:1770-1779. (CBA-216)
3. Choi, S.H. et al. (2014). MMP9 processing of HSPB1 regulates tumor progression. *PLoS One* **9**:e85509. (CBA-216)

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

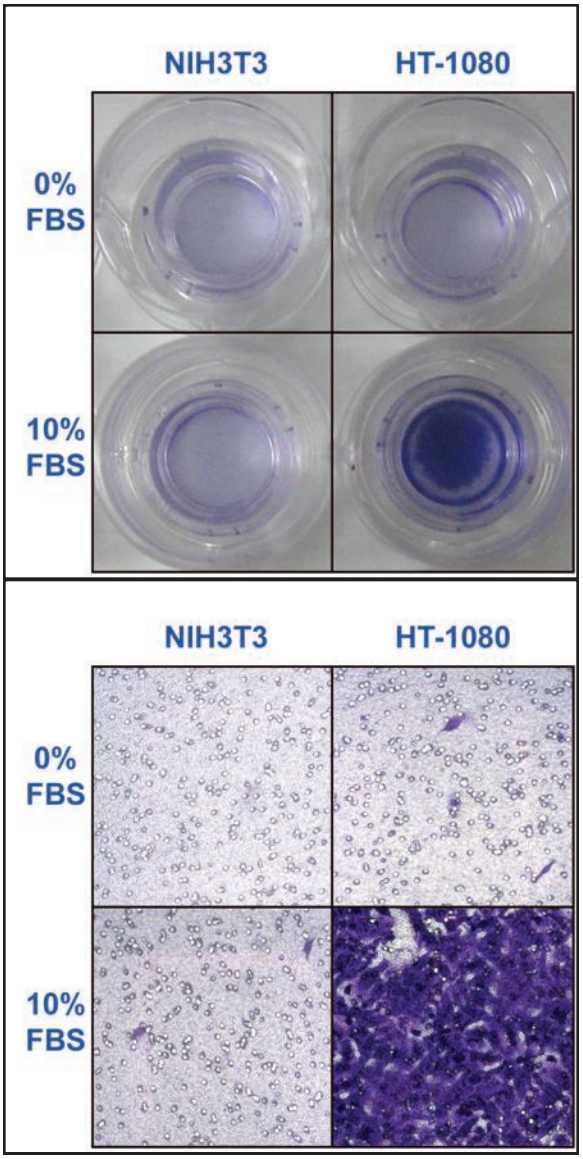
CytoSelect™ Cell Invasion Assays

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.



CytoSelect™ Cell Invasion Assay Principle.

- **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)

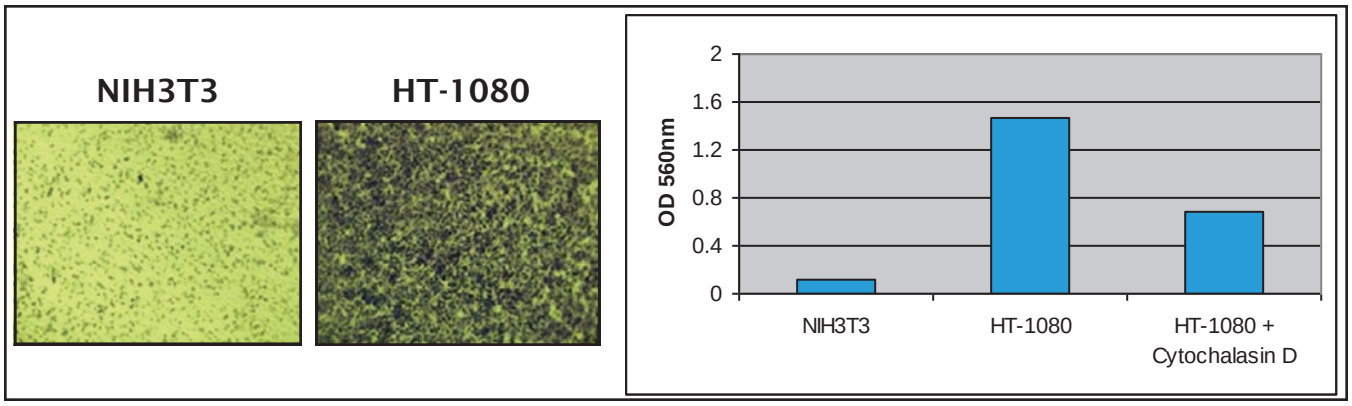


Human Fibrosarcoma HT-1080 Laminin I Cell Invasion. HT-1080 and NIH3T3 (negative control) were seeded at 200,000 cells/well and allowed to invade toward FBS for 24 hrs. Invasive cells on the membrane bottom were stained (top and center) and quantified at OD 560nm after extraction (data not shown).

Recent Product Citations

1. Osawa, Y. et al. (2015). Decreased expression of carbonyl reductase 1 promotes ovarian cancer growth and proliferation. *Int. J. Oncol.* **46**:1252-1258. (CBA-110)
2. Hirata, H. et al. (2015). Long noncoding RNA MALAT1 promotes aggressive renal cell carcinoma through Ezh2 and interacts with miR-205. *Cancer Res.* **75**:1322-1331. (CBA-110)
3. Cheng X. et al. (2015). LAPTM4B-35, a cancer-related gene, is associated with poor prognosis in TNM stages I-III gastric cancer patients. *PLoS One* **10**:e0121559. (CBA-110)
4. Chen, R. et al. (2015). The acetate/ACSS2 switch regulates HIF-2 stress signaling in the tumor cell microenvironment. *PLoS One* **10**:e0116515. (CBA-110)
5. Djuzenova, C.S. et al (2015). Actin cytoskeleton organization, cell surface modification and invasion rate of 5 gliobastoma cell lines differing in PTEN and p53 status. *Exp. Cell Res.* **330**:346-357. (CBA-110-COL)
6. Adam, M.G.. et al. (2015). SIAH ubiquitin ligases regulate breast cancer cell migration and invasion independent of the oxygen status. *Cell Cycle* **14**:3734-3747. (CBA-112)
7. Yamamoto, K. et al. (2014). miR-379/411 cluster regulates IL-18 and contributes to drug resistance in malignant pleural mesothelioma. *Oncol. Rep.* **32**:2365-2372. (CBA-112)
8. Takeuchi, S. et al. (2014). Significance of osteopontin in the sensitivity of malignant pleural mesothelioma to pemetrexed. *Int. J. Oncol.* **44**:1886-1894. (CBA-112)
9. Ho, P.W. et al. (2015). Knockdown of PTHR1 in osteosarcoma cells decreases invasion and growth and increases tumor differentiation in vivo. *Oncogene* **34**:2922-2933. (CBA-112-COL)

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

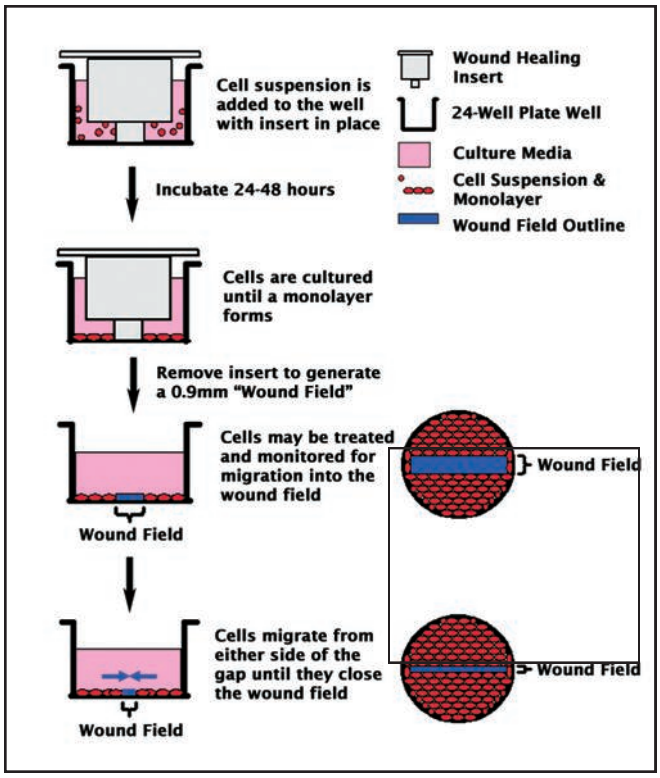
1. Yoo, B. et al. (2015). Combining miR-10b-targeted nanotherapy with low-dose doxorubicin elicits durable regressions of metastatic breast cancer. *Cancer Res.* **75**:4407-4415. (CBA-100-C)
2. Bhansali, M. et al. (2015). TM4SF3 and AR: A nuclear complex that stabilizes both proteins. *Mol. Endocrinol.* 10.1210/me.2015-1075. (CBA-101-C)
3. Zecchini, V. et al. (2015). Nuclear ARRB1 induces pseudohypoxia and cellular metabolism reprogramming in prostate cancer. *EMBO J.* **33**:1365-1382. (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
			2 x 60 Assays	CBA-100-C-5
		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

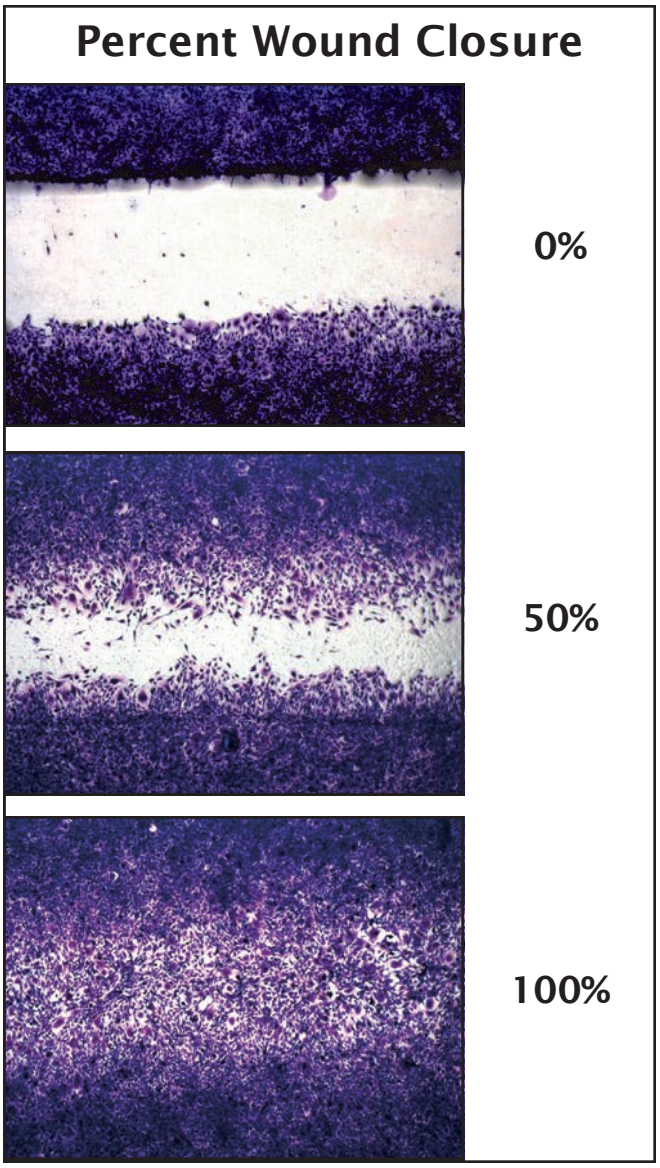
CytoSelect™ 24-Well Wound Healing / Cell Migration Assay

Compared to traditional scratch assays, 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.

- **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



CytoSelect™ 24-well Wound Healing Assay Principle.



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.

Recent Product Citations

1. Mazumder, A. et al. (2015). In vitro wound healing and cytotoxic effects of sinigrin-phytosome complex. *Int. J. Pharm.* **498**:283-293.
2. Widhe, M. et al. (2015). A fibronectin mimetic motif improves integrin mediated cell binding to recombinant spider silk matrices. *Biomaterials* **74**:256-266.
3. Delalande, A. et al. (2015). Enhanced Achilles tendon healing by fibromodulin gene transfer. *Nanomedicine* **11**:1735-1744.
4. Latifi-Pupovci, H. et al. (2015). In vitro migration and proliferation ("wound healing") potential of mesenchymal stromal cells generated from human CD271+ bone marrow mononuclear cells. *J. Transl. Med.* **13**:315.
5. Lakatos, K. et al. (2015). Mesenchymal stem cells respond to hypoxia by increasing diacylglycerols. *J. Cell Biochem.* 10.1002/jcb.25292.

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™ 24-Well Cell Co-Culture System

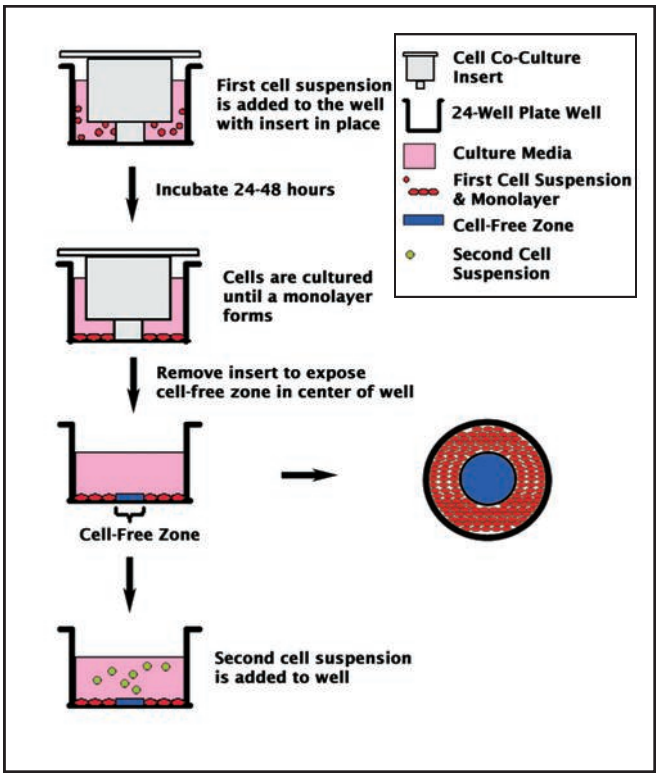
The culture of two cell lines together is advantageous for studying a variety of applications:

- Cell-cell interactions
- Cell activation
- Cellular differentiation
- Maintaining stem cell pluripotency
- Various effects of secreted factors from one cell type on a second cell type

Traditional methods of co-culture usually involve one of the following methods:

1. One cell type is cultured to form a monolayer, followed by seeding of a second cell type directly over the monolayer. This is a common method when feeder cells are used to maintain stem cells in an undifferentiated state. However, it is not useful when studying the effects of one cell on the other because the first cell is obscured from view by the second.
2. A Boyden Chamber (see page 12 for details) is used to culture one cell type above the membrane and a second cell type below the membrane. This system allows a separation between the two cells, but does not allow for direct cell-to-cell contact which may reduce its efficacy for certain applications.

The CytoSelect™ Cell Co-Culture System provides a unique platform for direct contact between two cell types in one well. A proprietary molded plastic insert creates a cell-free zone in the center of a 24-well cell culture-treated plate. The first cell type is seeded in the area around the insert. Once the cells form a monolayer, the insert is removed and the second cell is seeded.



Protocol for the CytoSelect™ 24-Well Co-Culture System. Cell type #1 is seeded with the Co-Culture insert in place. After cells form a monolayer, the insert is removed and cell type #2 is seeded.

Product Name	Detection	Size	Catalog Number
CytoSelect™ 24-Well Cell Co-Culture System	Microscopy	24 Assays	CBA-160
		5 x 24 Assays	CBA-160-5

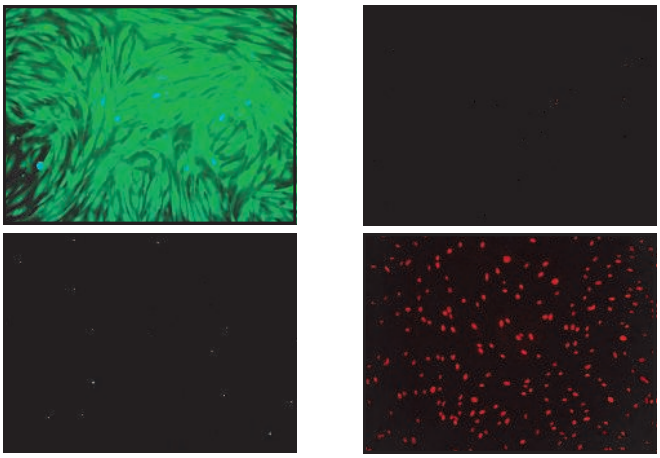
CytoSelect™ Cell Viability and Cytotoxicity Assay (Live/Dead Cells)

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 by microscopy, colorimetric or fluorescence plate reader, or flow cytometry
- **Quantitative**: Quantify cells on a colorimetric or fluorescence plate reader

Recent Product Citations

1. Hermann, D.M. et al. (2015). Sustained neurological recovery induced by resveratrol is associated with angioneurogenesis rather than neuroprotection after focal cerebral ischemia. *Neruobiol. Dis.* **83**:16-25.
2. Maity, G. et al. (2015). Aspirin blocks growth of breast tumor cells and tumor-initiating cells and induces reprogramming factors of mesenchymal to epithelial transition. *Lab Invest.* 10.1038/labinvest.2015.49.
3. Wu, M.Y. et al (2015). MiR-34a regulates therapy resistance by targeting HDAC1 and HDAC7 in breast cancer. *Cancer Lett.* **354**:311-319.



Viability of Human Foreskin Fibroblasts. BJ-TERT cells were allowed to culture for 24 hours, and 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.

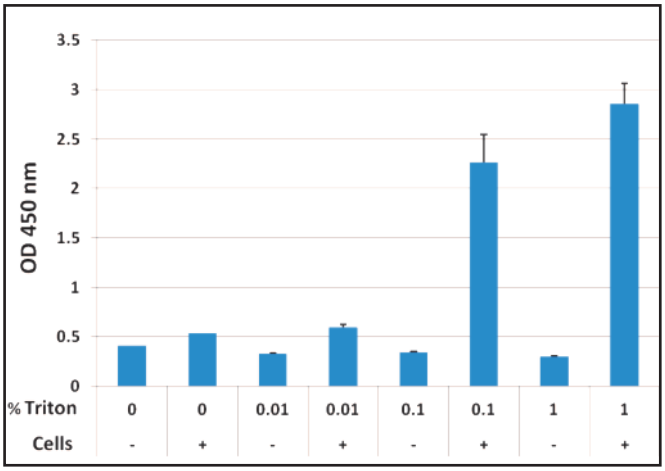
Product Name	Detection	Size	Catalog Number
CytoSelect™ Cell Viability and Cytotoxicity Assay Kit	Colorimetric / Fluorometric	96 Assays	CBA-240

CytoSelect™ LDH Cytotoxicity Assay

Loss of cell membrane integrity is one of the hallmarks of cytotoxicity. Upon cell death, lactate dehydrogenase (LDH) is released from the cytoplasm through the damaged membrane. Our CytoSelect™ LDH Cytotoxicity Assay provides a convenient plate-based method for testing cytotoxicity based on LDH release. In this assay, cells are cultured in a 96-well plate with and without the compound to be tested. LDH released into the media from cells converts a lactate substrate to pyruvate and generates NADH. In the presence of NADH, the colorimetric dye WST-1 is converted to a formazan that generates an orange color which is detected in a colorimetric plate reader.

Recent Product Citations

1. Chu, J. et al. (2015). Gamma secretase-activating protein is a substrate for caspase-3: implications for Alzheimer's disease. *Biol. Psychiatry* **77**:720-728.
2. Li, J.G. et al. (2014). Homocysteine exacerbates ß-amyloid pathology, tau pathology, and cognitive deficit in a mouse model of Alzheimer's disease with plaques and tangles. *Ann. Neurol.* **75**:851-863.



LDH Release from HEK 293 Cells. 20,000 cells/well were cultured for 24 hours. After adding various concentrations of Triton X-100, the LDH Cytotoxicity Assay Reagent was added followed by a 30 minute incubation at 37ºC and 5% CO2.

Product Name	Detection	Size	Catalog Number
CytoSelect™ LDH Cytotoxicity Assay Kit	Colorimetric	960 Assays	CBA-241

Cellular Senescence Assays

Senescence Associated β-galactosidase is a common biochemical marker of cellular senescence. SA ß-Gal produces a blue color in senescent cells. We offer three kit formats to test cellular senescence via SA-ß-galalactosidase activity:

- Our **ß-Gal Staining Kit** allows you to visualize senescence by standard light microscope.
- Our **Quantitative Cellular Senescence Assay** measures senescence in cells cultured in a 35mm dish by either flow cytometry or fluorescence microscopy
- Our **96-Well Cellular Senescence Assay** provides a higher throughput assay in a fluorescence plate reader.

Recent Product Citations

1. Gan, W. et al. (2015). ERK5/HDAC5-mediated, resveratrol-, and pterostilbene-induced expression of MnSOD in human endothelial cells. *Mol. Nutr. Food Res.* 10.1002/mnfr.201500466. (CBA-230)
2. Lee, D. H. et al. (2014). Synergistic effect of JQ1 and rapamycin for treatment of human osteosarcoma. *Int. J. Cancer* **136**:2055-2064. (CBA-230)
3. Chang, Z. et al. (2015). Ascorbic acid provides protection for human chondrocytes against oxidative stress. *Mol. Med. Rep*. 10.3892/mmr.2015.4231. (CBA-231)
4. Hu, W. et al (2015). Mechanistic investigation of bone marrow suppression associated with palbociclib and its differentiation from cytotoxic chemotherapies. *Clin. Cancer Res.* 10.1158/1078-0432-CCR-15-1421. (CBA-231 and CBA-232)
5. Kim, J. et al. (2014). p53 induces skin aging by depleting Blimp1+ sebaceous gland cells. *Cell Death Dis.* **5**:e1141. (CBA-232)

Product Name	Detection	Size	Catalog Number
Cellular Senescence Assay Kit (SA β-gal Staining)	Light Microscopy	50 Assays	CBA-230
		5 x 50 Assays	CBA-230-5
96-Well Cellular Senescence Assay (SA β-gal Activity)	Fluorometric Plate Reader	120 Assays	CBA-231
		5 x 120 Assays	CBA-231-5
Quantitative Cellular Senescence Assay (SA β-gal)	Flow Cytometry / Fluorescence Microscopy	10 Assays	CBA-232
		5 x 10 Assays	CBA-232-5

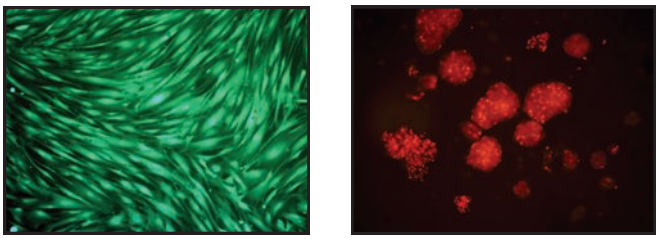
CytoSelect™ Anoikis Assays

Anoikis is defined as death of adherent cells due to loss of adhesion to the extracellular matrix. Our Anoikis Assays allow you to quantify and monitor anchorage-dependent cell death using a 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 an EthD-1 reagent.

Recent Product Citations

1. Lee, H.W. et al. (2013). Tpl2 kinase impacts tumor growth and metastasis of clear cell renal cell carcinoma. *Mol. Cancer Res.* **11**:1375-1386. (CBA-080)
2. 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)
3. 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)

- **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.

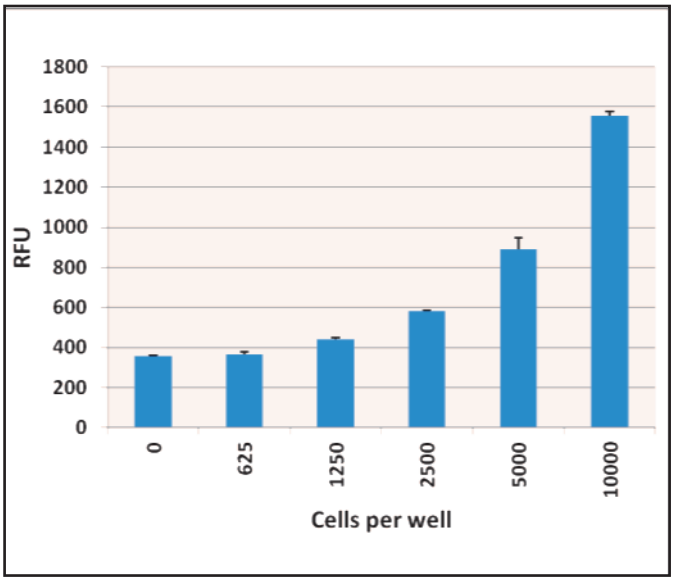
Product Name	Detection	Size	Catalog Number
CytoSelect™ 24-Well Anoikis Assay	Colorimetric / Fluorometric	24 Assays	CBA-080
CytoSelect™ 96-Well Anoikis Assay	Colorimetric / Fluorometric	96 Assays	CBA-081

CytoSelect™ Fluorometric Cell Proliferation Assay Reagent

Cell proliferation is easily measured by the addition of a variety of dyes that can be correlated with the number of cells. Various dyes producing a visible color are available to measure proliferation rates, but fluorometric dyes are often more sensitive and may be a superior choice for researchers with access to a fluorescence-based microplate reader.

Our CytoSelect™ Cell Proliferation Assay Reagent (Fluorometric) provides a simple, single reagent method to measure proliferation of cells. The fluorometric dye is added directly to cultured cells. Upon entering metabolically active live cells, the non-fluorescent dye is converted to a bright red fluorescent form. Quantitation is performed using a fluorescence plate reader with excitation at 560 nm and emission at 590-600 nm.

This reagent is versatile and can be used with a wide variety of cell types including cultured mammalian and piscine cells, bacteria, yeast, fungi, and protozoa.



Human HEK 293 Cell Density. Cells were seeded at various densities in triplicate and allowed to culture for 24 hours. Cells were then treated with the CytoSelect™ Cell Proliferation Assay Reagent for 6 hours at 37ºC and 5% CO2.

Product Name	Detection	Size	Catalog Number
CytoSelect™ Cell Proliferation Assay Reagent, Fluorometric	Fluorometric	960 Assays	CBA-250

CytoSelect™ MTT Cell Proliferation Assay

Cell proliferation is easily measured by the addition of a variety of dyes that produce a visible color that can be correlated with the number of cells. Our CytoSelect™ MTT Cell Proliferation Assay provides a simple method to measure proliferation of cells. The cell-permeable MTT dye is added directly to cultured cells followed by a detergent solution. Quantitation is performed using a standard microplate reader at 540-570 nm.

Recent Product Citations

1. Dogan, M. et al. (2015). Are the leading drugs against *Staphylococcus aureus* really toxic to cartilage? *J. Infect. Public Health* 10.1016/j.jiph.2015.10.004.
2. Wu, H. et al. (2015). MicroRNA-21 is a potential link between non-alcoholic fatty liver disease and hepatocellular carcinoma via modulation of the HBP1-p53-Srebp1c pathway. *Gut* 10.1136/gutjnl-2014-308430.
3. Ren, Z. et al. (2014). Anti-tumor effect of a novel soluble recombinant human endostatin: administered as a single agent or in combination with chemotherapy agents in mouse tumor models. *PLoS One* **9**:e107823.

Product Name	Detection	Size	Catalog Number
CytoSelect™ MTT Cell Proliferation Assay	Colorimetric	960 Assays	CBA-252

CytoSelect™ WST-1 Cell Proliferation Assay Reagent

Our CytoSelect™ WST-1 Cell Proliferation Assay provides a similar method to our MTT Cell Proliferation Assay, but with a single reagent format that does not require a detergent solubilization step. Quantitation is performed using a standard microplate reader at 450 nm.

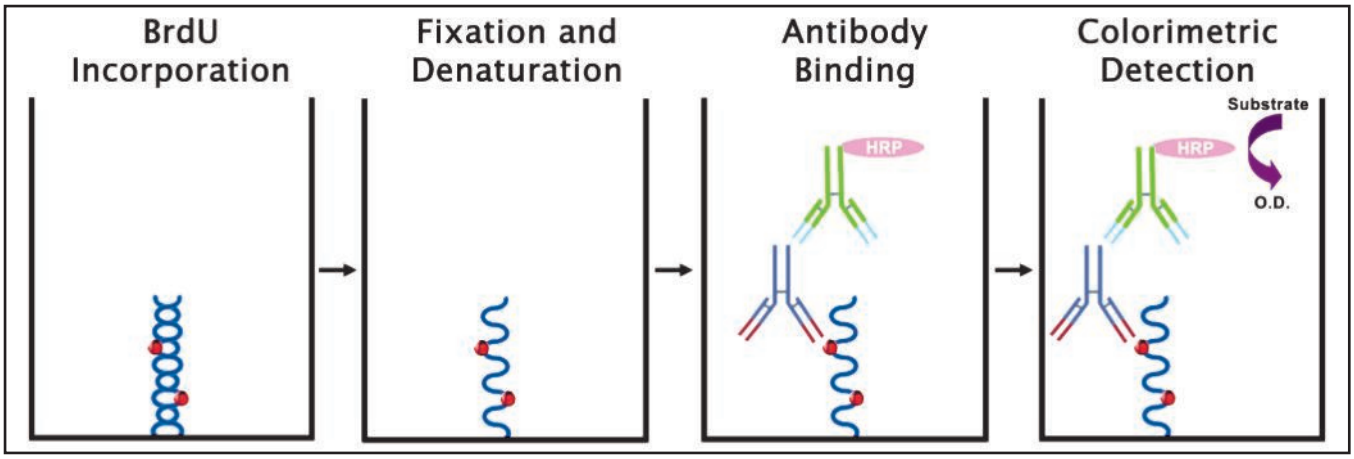
Product Name	Detection	Size	Catalog Number
CytoSelect™ WST-1 Cell Proliferation Assay Reagent	Colorimetric	960 Assays	CBA-253

CytoSelect™ BrdU Cell Proliferation ELISA Kit

BrdU is a thymidine analog that can incorporate into newly synthesized DNA strands of actively proliferating cells. Our CytoSelect™ BrdU Cell Proliferation ELISA Kit provides a convenient plate-based method to measure this incorporation. Once the BrdU is incorporated into the DNA, cells are fixed and DNA is denatured. Incorporated BrdU can be quantified in the denatured DNA by an anti-BrdU antibody.

Recent Product Citations

1. Kreiseder, B. et al. (2015). Alpha-catulin contributes to drug-resistance of melanoma by activating NF-kappaB and AP-1. *PLoS One* **10**:e0119402.
2. Hatzis, C. et al. (2014). Enhancing reproducibility in cancer drug screening: how do we move forward? *Cancer Res.* **74**:4016-4023.



Assay Principle for the CytoSelect™ BrdU Cell Proliferation ELISA Kit.

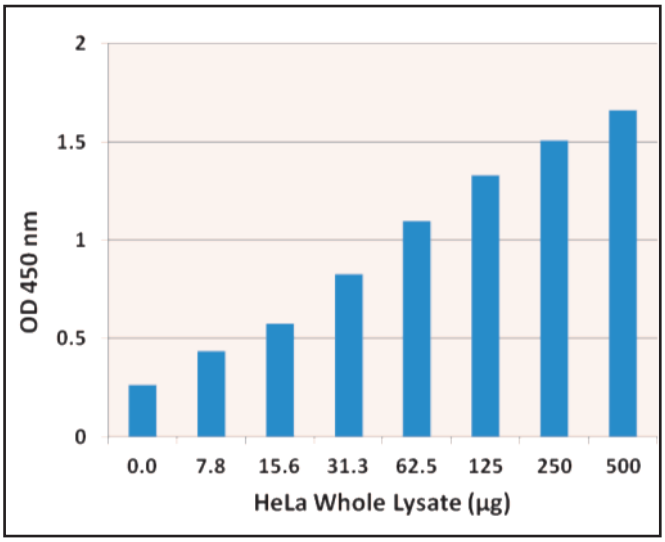
Product Name	Detection	Size	Catalog Number
CytoSelect™ BrdU Cell Proliferation ELISA Kit	Colorimetric	96 Assays	CBA-251

CytoSelect™ Proliferating Cell Nuclear Antigen (PCNA) ELISA Kit

Proliferating Cell Nuclear Antigen (PCNA) acts as a processivity factor for DNA polymerase by associating with various proteins involved in DNA replication. It is also associated with chromatin remodeling and cell cycle control, and it is often used as a marker of cell proliferation.

Our CytoSelect™ PCNA ELISA Kit provides a convenient plate-based method to quantify PCNA levels in nuclear or whole cell extracts.

- **Sensitive**: Detect PCNA as low as 12.5 ng/mL
- **Versatile**: Measure PCNA levels from human, mouse, or rat whole cell lysates or nuclear extracts
- **Quantitative**: Measure results in a colorimetric plate reader against a provided PCNA standard



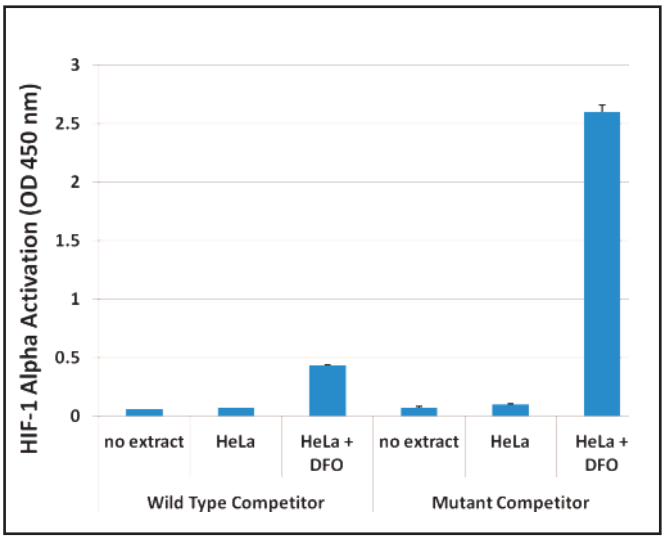
PCNA Detection in HeLa Whole Cell Lysates. Whole cell lysates were prepared in a RIPA lysis buffer. Protein concentrations were determined by BCA protein assay.

Product Name	Detection	Size	Catalog Number
CytoSelect™ Proliferating Cell Nuclear Antigen (PCNA) ELISA Kit	Colorimetric	96 Assays	CBA-254

HIF-1 Alpha DNA Binding Activity Assay Kit

Cell hypoxia, or low oxygen condition, is a normal physiological response to certain body stressors such as high altitudes, but it can also be a symptom of pathological conditions and is often used as a marker for tumor cells. In response to hypoxic conditions, the hypoxia-inducible factor 1 transcriptional activator complex (HIF-1) plays a role in activating several hypoxia-responsive genes such as erythropoietin and VEGF. During hypoxia, the alpha subunit of HIF-1 accumulates and translocates from the cytosol to the nucleus, where it dimerizes with the beta subunit and becomes transcriptionally active. It then binds transcriptional coactivators to induce gene expression.

The HIF-1 Alpha DNA Binding Activity Assay Kit detects activated HIF-1 in an ELISA format. Active HIF-1 complex is captured on a double-stranded oligo containing a hypoxic response element (HRE) that is attached to the plate. Detection is then performed with a primary antibody followed by an HRP-conjugated secondary antibody. The assay will detect HIF-1 complexes from human, mouse or rat protein samples.



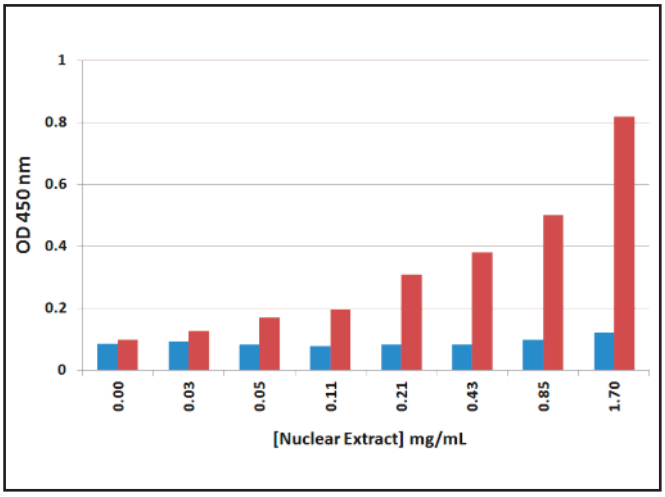
Detection Specificity of HIF-1 Alpha. HeLa cells were incubated in the presence or absence of 0.2 mM deferoxamine mesylate (DFO) for 4 hours at 37ºC. Nuclear extracts were prepared using the Nuclear/Cytosolic Fractionation Kit (#AKR-171). 100 pmol of non-biotinylated wild type or mutated HRE double stranded competitor oligos were added to the Complete DNA Binding Buffer just prior to inclusion in the assay.

Product Name	Detection	Size	Catalog Number
HIF-1 Alpha DNA Binding Activity Assay Kit	Colorimetric	96 Assays	CBA-282

HIF-1 Alpha ELISA Kits

Our HIF-1 Alpha ELISA Kits provide a convenient method for detection and quantitation of human, mouse, or rat HIF-1 Alpha in cells or tissues. Two ELISA kit formats are available:

- The HIF-1 Alpha Sandwich ELISA Kit detects HIF-1 Alpha in any protein sample including tissue homogenates, whole cell lysates, or nuclear extracts. Samples are added to an anti-HIF-1 Alpha antibody coated plate. Quantitation of unknown samples is performed by comparison of the OD values to those of a known standard.
- The HIF-1 Alpha Cell Based ELISA Kit allows the detection of HIF-1 Alpha levels in intact cells. Cells are seeded in a tissue culture treated plate suitable for reading in a 96-well plate-based luminometer. Cells are fixed and permeabilized to allow detection with the anti-HIF-1 antibody. Detection is performed by chemiluminescence.



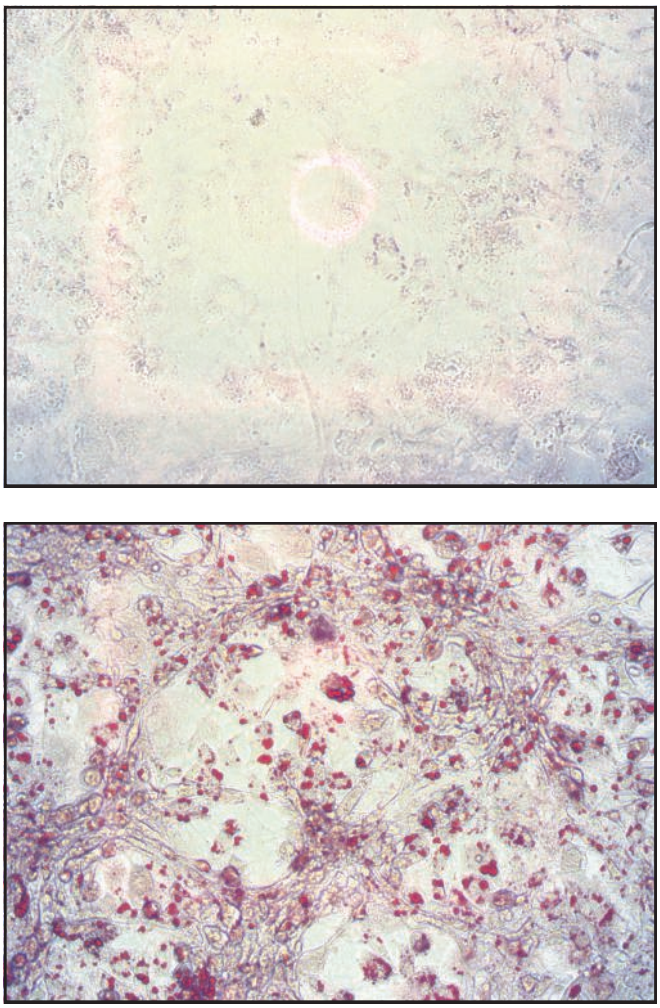
Detection of Nuclear HIF-1 Alpha with the HIF-1 Alpha Sandwich ELISA Kit. HeLa cells were incubated in the presence or absence of 0.2 mM DFO for 4 hours at 37ºC. HIF-1 Alpha levels were measured in untreated (blue bars) and treated (red bars) nuclear extracts according to the Assay Protocol.

Product Name	Detection	Size	Catalog Number
HIF-1 Alpha Sandwich ELISA Kit	Colorimetric	96 Assays	CBA-280
HIF-1 Alpha Cell Based ELISA Kit	Chemiluminescent	96 Assays	CBA-281

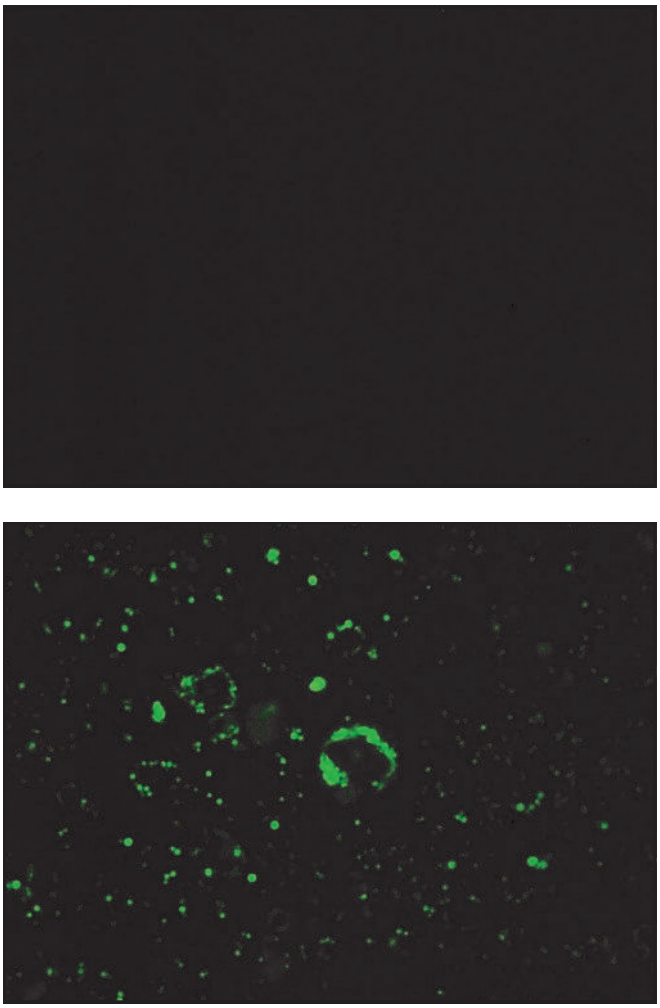
CytoSelect™ 96-well Adipogenesis Assay Kit

The ability to regulate the cell cycle and differentiation of adipocytes is important to the understanding of obesity. Adipogenesis is the process in which preadipocytes develop into mature adipocytes in a multistep process that requires the sequential activation of numerous transcription factors. The 3T3-L1 cell line is the best characterized model for adipogenesis *in vitro*. 3T3-L1 cells display a fibroblast-like phenotype when grown under normal conditions. However, when treated with a combination of IBMX, insulin, and dexamethasone, these cells undergo terminal differentiation resulting in a more rounded phenotype and the formation of intracellular lipid droplets.

The CytoSelect™ 96-Well Adipogenesis Assay quantitatively measures lipid droplet accumulation in cultured cells of the 3T3-L1 model. Quantitation is performed either in a standard colorimetric plate reader with Oil Red O stain, or in a fluorescence plate reader with Nile Red fluorometric stain.



Staining of 3T3-L1 Cells with Oil Red O. 20,000 cells/well of preadipocyte 3T3-L1 cells were seeded overnight in a 96-well plate. Cells were uninduced (top) or induced (bottom) for 7 days and stained with Oil Red O colorimetric stain according to the Assay Protocol.



Staining of 3T3-L1 Cells with Nile Red Fluorescent Stain. 20,000 cells/well of preadipocyte 3T3-L1 cells were seeded overnight in a 96-well plate. Cells were uninduced (top) or induced (bottom) for 7 days and stained with Nile Red Fluorescent Stain according to the Assay Protocol.

Product Name	Detection	Size	Catalog Number
CytoSelect™ 96-Well Adipogenesis Assay Kit	Colorimetric / Fluorometric	200 Assays	CBA-290

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.

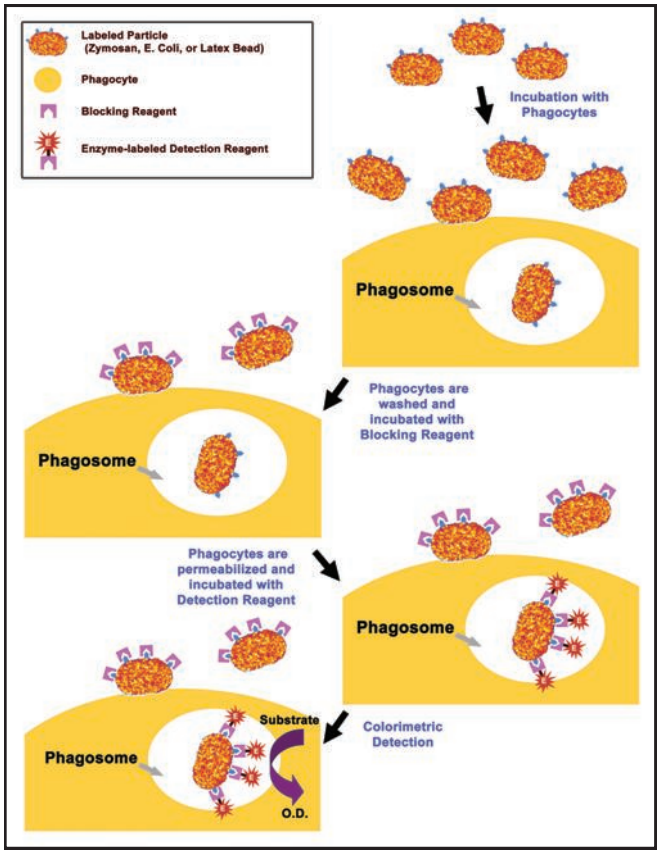
CytoSelect™ 96-Well Phagocytosis Assays are more accurate, high-throughput alternatives to the standard phagocytosis assay. The assays may be adapted for use in 48-well and 24-well plates if desired.

- **Highly Accurate**: Eliminates manual counting
- **High Throughput**: 96-well plate format
- **Quantitative**: Measure OD in a standard microplate reader
- **Flexible**: Choose from 3 substrates: E. coli, Zymosan particles, or red blood cells*

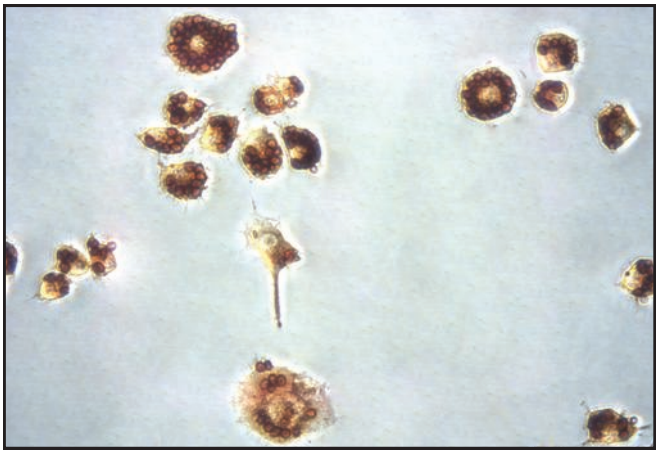
*Red blood cells are not provided in the kit. Fresh RBCs should be obtained immediately prior to running the assay. E. coli and Zymosan particles are provided in their respective kits.

Recent Product Citations

1. Yu, Z. et al. (2015). Therapeutic concentration of lithium stimulates complement C3 production in dendritic cells and microglia vis GSK-3 inhibition. *Glia* **63**:257-270. (CBA-220)
2. Park, S.Y. et al. (2014). Immunostimulatory effect of fermented red ginseng in the mouse model. *Prev. Nutr. Food Sci.* **19**:10-18. (CBA-220)
3. Martin, I. et al. (2015). Fasciola hepatica fatty acid binding protein inhibits TLR4 activation and suppresses the inflammatory cytokines induced by lipopolysaccharide in vitro and in vivo. *J. Immunol.* 10.4049/jimmunol.1401182. (CBA-222)
4. Zhu, X. et al. (2014). Deletion of class A scavenger receptor deteriorates obesity-induced insulin resistance in adipose tissue. *Diabetes* **63**:562-577. (CBA-222)
5. Lee, S.G. et al. (2015). Immunostimulatory polysaccharide isolated from the leaves of Diospyros kaki Thumb modulate macrophage via TLR2. *Int. J. Biol. Macromol.*. **79**:971-982. (CBA-224)
6. Zhang, H. et al. (2015). Functional analysis and transcriptomic profiling of iPSC-derived macrophages and their application in modeling Mendelian disease. *Circ. Res.* 10.1161/CIRCRESAHA.117.305860. (CBA-224)



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 (E. coli)	Colorimetric	96 Assays	CBA-222
CytoSelect™ 96-Well Phagocytosis Assay (Red Blood Cell)	Colorimetric	96 Assays	CBA-220
CytoSelect™ 96-Well Phagocytosis Assay (Zymosan)	Colorimetric	96 Assays	CBA-224
		5 x 96 Assays	CBA-224-5

CytoSelect™ Cell Contraction Assay Kits (Floating Matrix Model)

The wound healing process 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.

Various culture models are available to study the ability of fibroblasts to reorganize and contract collagen matrices in vitro. In the floating matrix model, a freshly polymerized collagen matrix containing cells is released from the culture dish and allowed to float in culture medium. Contraction occurs in the absence of external mechanical load and without appearance of stress fibers in the cells.

The CytoSelect™ Cell Contraction Assay Kits (Floating Matrix Model) provide a simple system to assess cell contractivity and to screen for cell contraction mediators. The proprietary Cell Contraction Plate eliminates the matrix releasing step of the conventional attached model assay, providing a faster, higher-throughput method to assess cell contraction. Kits are available in 24-well and 48-well formats.

Contraction Inhibition by BDM. 5 x 10^5 COS-7 cells in 0.5 mL collagen gel lattice were cultured for two days according to the Assay Protocol. Dashed lines indicate the gel edges.

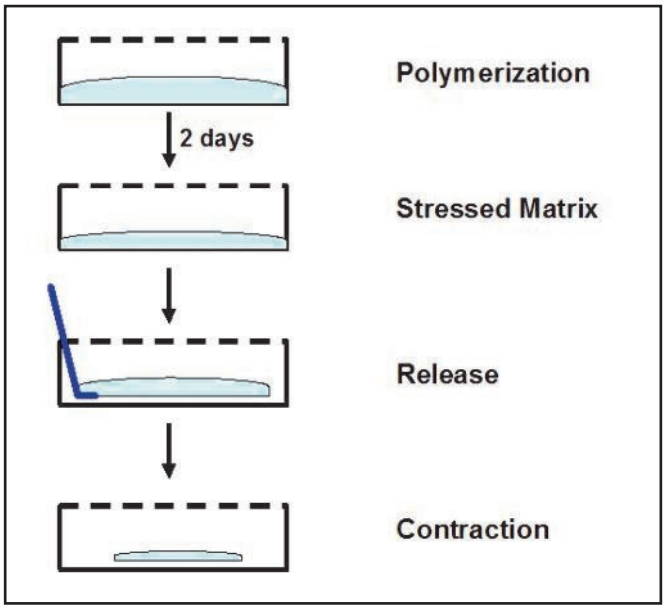
Product Name	Detection	Size	Catalog Number
CytoSelect™ 24-Well Cell Contraction Assay	Light Microscopy	24 Assays	CBA-5020
CytoSelect™ 48-Well Cell Contraction Assay	Light Microscopy	48 Assays	CBA-5021

Collagen-Based Cell Contraction Assay (Two-Step Attached Model)

Our Cell Contraction Assay (Attached Model) 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 Citations

1. Ye, Y. et al. (2016). Down-regulation of 14-3-3 Zeta inhibits TGF-ß1-induced actomyosin contraction in human trabecular meshwork cells through RhoA signaling pathway. *Invest. Ophthalmol. Vis. Sci.* **57**:71.
2. Rinella, L. et al. (2016). Extracorporeal shockwaves modulate myofibroblast differentiation of adipose-derived stem cells. Wound Repair Regen. 10.1111/wrr.12410.
3. Halim, D. et al. (2015). ACTG2 variants impair actin polymerization in sporadic Megacystis Microcolon Intestinal Hypoperistalsis Syndrome. *Hum. Mol. Genet.* 10.1093/hmg/ddv497.
4. Li, H.Y. et al. (2015). Activation of TGF-ß1-CD147 positive feedback loop in hepatic stellate cells promotes liver fibrosis. *Sci. Rep.***5**:16552.
5. Duru, N. et al. (2015). NRF2/miR-140 signaling confers radioprotection to human lung fibroblasts. *Cancer Lett.* 10.1016/j.canlet.2015.08.011.
6. Gutierrez, J. et al. (2015). RECK-mediated ß1-integrin regulation by TGF-ß1 is critical for wound contraction in mice. *PLoS One* **10**:e0135005.



Assay Principle for the Collagen-Based Cell Contraction Assay (Attached Model).

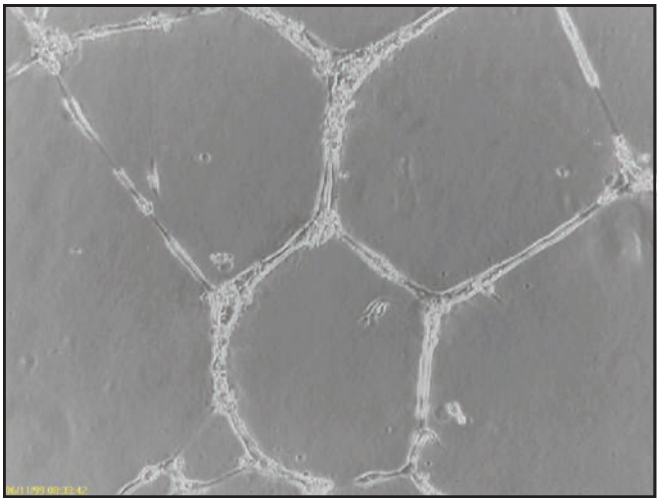
Product Name	Detection	Size	Catalog Number
Cell Contraction Assay	Light Microscopy	24 Assays	CBA-201

Endothelial Tube Formation (*In Vitro* Angiogenesis) Assay

For angiogenesis to occur, endothelial cells must escape their stable location and break through the basement membrane. 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. Zheng, D. et al. (2015). Silencing of miR-195 reduces diabetic cardiomyopathy in C57BL/6 mice. *Diabetologia* 10.1007/s00125-015-3622-8.
2. Bae, W. J. et al. (2015). Effects of sodium tri-and hexametaphosphate on proliferation, differentiation, and angiogenic potential of human dental pulp cells. *J. Endod.* 10.1016/j.joen.2015.01.038.
3. Zhang, J. et al. (2015). Effects of bioactive cements incorporating zinc-bioglass nanoparticles on odontogenic and angiogenic potential of human dental pulp cells. *J. Biomater. Appl.* **29**:954-964.
4. Yamanegi, K. et al. (2015). Sodium valproate, a histone deacetylase inhibitor, modulates the vascular endothelial growth inhibitor-mediated cell death in human osteosarcoma and vascular endothelial cells. *Int. J. Oncol.* 10.3892/ijo.2015.2924.



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

Our GFP-LC3 expression vectors are convenient tools for the study of autophagy. These vectors are available in three formats: mammalian, lentiviral, and retroviral expression vectors. Each vector contains a GFP reporter gene. A GFP control plasmid is provided at no additional charge.

Recent Product Citations

1. Yao, F. et al. (2015). Apelin-13 impedes foam cell formation by activating class III PI3K/Beclin-1-mediated autophagic pathway. *Biochem. Biophys. Res. Commun.* 10.1016/j.bbrc.2015.09.045. (CBA-401)
2. Pi, H. et al. (2015). SIRT3-SOD2-mROS-dependent autophagy in cadmium-induced hepatotoxicity and salvage by melatonin. *Autophagy* **11**:1037.1051. (CBA-401)
3. He, Z. et al. (2015). Atorvastatin induces autophagic cell death in prostate cancer cells in vitro. *Mol. Med. Rep.* 10.3892/mmr.2015.3334. (CBA-401)
4. Li, B.H. et al. (2014). TRPV1 activation impedes foam cell formation by inducing autophagy in oxLDL-treated vascular smooth muscle cells. *Cell Death Dis*. **5**:e1182. (CBA-401)
5. Meng, X. et al. (2014). Attenuation of Aß254-35-induced parallel autophagic and apoptotic cell death by gypenoside XVII through the estrogen receptor-dependent activation of Nrf2/ARE pathways. *Toxicol. Appl. Pharmacol*. **279**:63-75. (CBA-401)
6. Chen, W. et al. (2012). Andrographolide induces autophagic cell death in human liver cancer cells through cyclophilin D-mediated mitochondrial permeability transition pore. *Carcinogenesis* 10.1093/carcin/bgs264. (CBA-401)
7. Cina, D.P. et al. (2012). Inhibition of MTOR disrupts autophagic flux in podocytes. *J. Am. Soc. Nephrol.* **23**:412-420. (CBA-401)
8. 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

Biogenuix Medsystems Pvt. Ltd.
412-B, Jyoti Shikhar Building, District Center,
Janakpuri, New Delhi-110058, INDIA
Phone : +91-11-4875-4875
E-mail : contact@biogenuix.com
Web : www.biogenuix.com