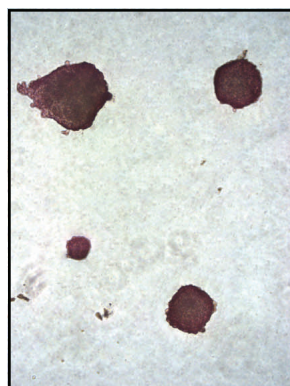
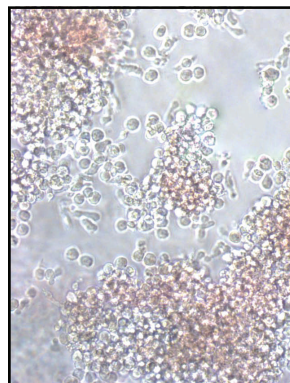
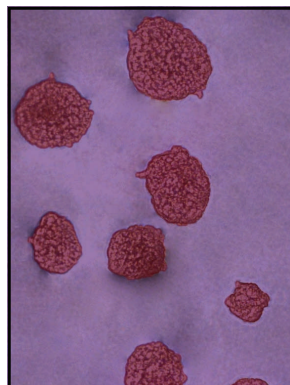


# Cell Colony Formation Assays



Choose your Colony Formation Assay		
Cell Type	Medium	Assay
Tumor Cells	Soft Agar Matrix	Cell Transformation Assays <b>(p. 2-3)</b>
		<i>In Vitro</i> Tumor Sensitivity Assay <b>(p. 4)</b>
		Clonogenic Tumor Cell Isolation Kit <b>(p. 5)</b>
Hematopoietic Cells	Methylcellulose	Hematopoietic Colony Forming Cell Assay <b>(p. 6)</b>
Embryonic Stem Cells	Soft Agar Matrix	Stem Cell Colony Formation Assay <b>(p. 7)</b>

**Advanced, high-throughput soft agar assays with more accurate quantitation in substantially less time**

## Cell Transformation Assays

The traditional soft agar colony formation assay has been used to measure cell transformation and anchorage-independent growth, with manual counting of proliferated cells after 3-4 weeks of cell growth.

We have advanced the soft agar assay with unique improvements that provide increased throughput and more accurate quantitation, while reducing the assay time by more than 50%.

Our CytoSelect™ Cell Transformation Assays are available in a variety of formats, all of which offer the same unique benefits:

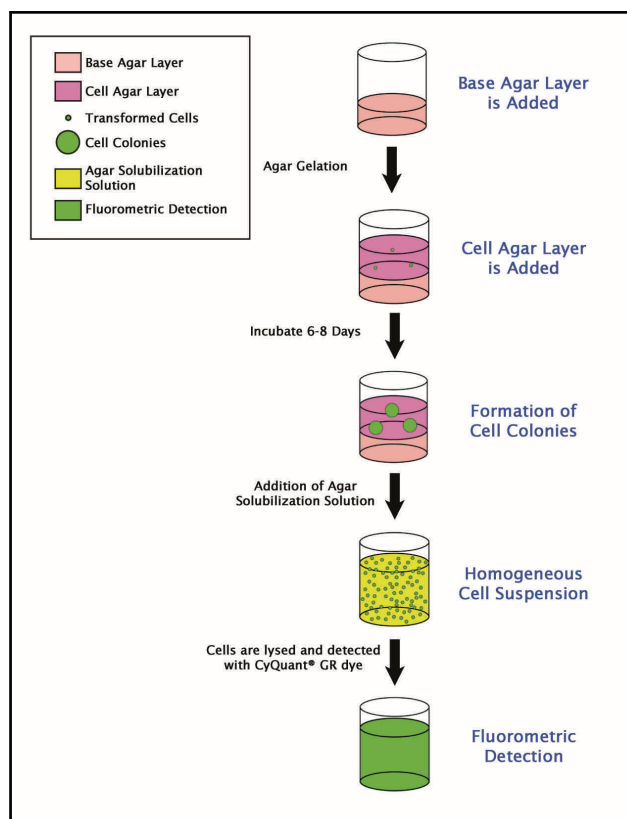
- **More Accurate Quantitation:** our assays require no manual cell counting
- **Faster Results:** quantify your cell colonies in just 6-8 days, not 3-4 weeks
- **Higher Throughput:** our assays may be run in 96-well or 384-well plates, although the protocol may be adapted to virtually any plate format

### 96-Well Cell Transformation Assay (Soft Agar Colony Formation)

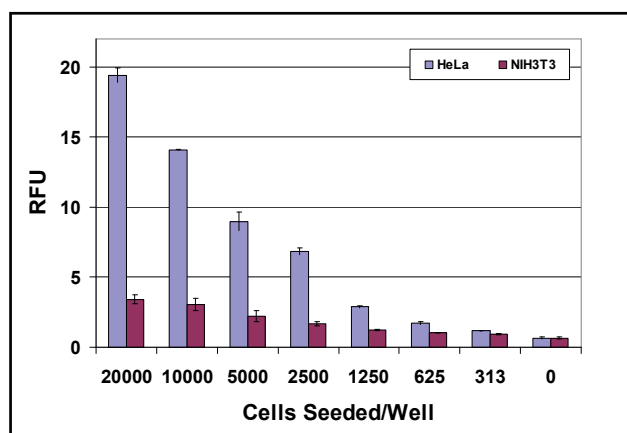
Our original CytoSelect™ Cell Transformation Assay was developed to improve both the speed and accuracy of the traditional soft agar assay. This assay is now widely used in both academic and industrial research laboratories worldwide.

In this assay, cells are incubated in a semisolid agar matrix for about one week, then solubilized and lysed (see Assay Principle, right). Quantitation of cell colonies is performed using the fluorescent dye CyQuant® GR in a fluorometric plate reader.

This assay is ideal for quantitation of transformed cells where no downstream analysis is required. If you need to recover your colonies, use our Cell Recovery Compatible assay (next page).



**Assay Principle for the CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation).**



**Anchorage-Independent Growth of HeLa and NIH3T3 Cells Quantified after 6 Days with the CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation).**

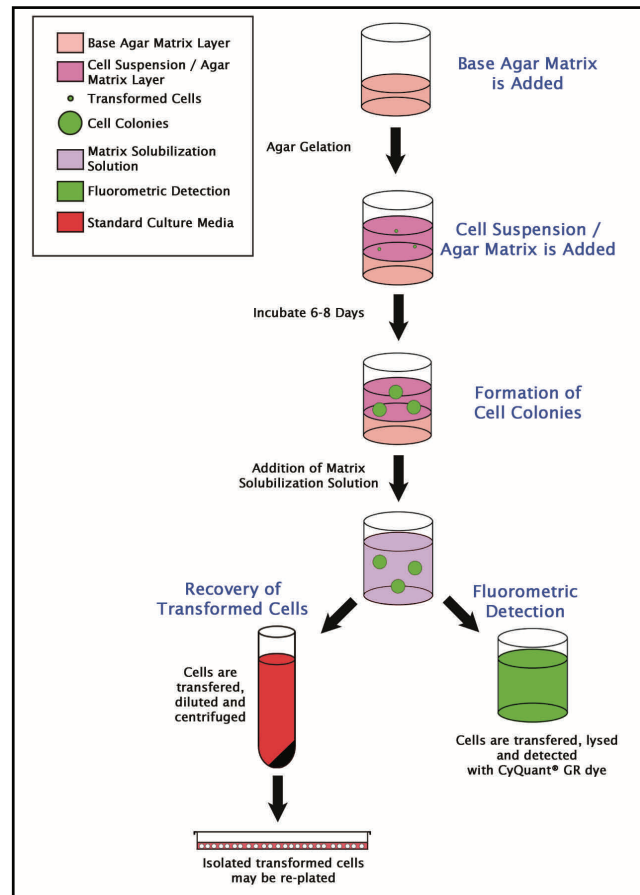
## 96-Well Cell Transformation Assay (Cell Recovery Compatible)

While our original Cell Transformation Assay (previous page) is a powerful advancement on the traditional soft agar assay, it does not allow recovery of cell colonies from the matrix. After diligent research efforts, we developed a modified soft agar formulation that allows cells to be recovered intact and viable, allowing further downstream analysis.

With our CytoSelect™ 96-Well Cell Transformation Assay (Cell Recovery Compatible), cells are incubated in the proprietary semisolid agar matrix for about one week. Cells can then be either solubilized, lysed and quantified in a fluorometric plate reader, or recovered from the 3D culture medium (see Assay Principle, right). Running samples in duplicate allows you to quantify your sample in one well and recover cells from another.

## 384-Well Cell Transformation Assay

Our CytoSelect™ 384-Well Cell Transformation Assay uses the same proprietary semisolid agar medium used in our Cell Recovery Compatible assay above. Due to the small well size of 384-well plates, it is not recommended to attempt cell recovery with this assay.



**Assay Principle for the CytoSelect™ 96-Well Cell Transformation Assay (Cell Recovery Compatible).**

### Cell Transformation Assay Selection Guide

Assay	Recover Cells for Further Analysis	Detection Method	Kit Sizes*
<b>96-Well Cell Transformation Assay (Soft Agar Colony Formation)</b>	No	Fluorometric	1 Plate 5 Plates
<b>96-Well Cell Transformation Assay (Cell Recovery Compatible)</b>	Yes	Colorimetric	1 Plate 5 Plates
		Fluorometric	1 Plate 5 Plates
<b>384-Well Cell Transformation Assay</b>	No	Fluorometric	384 wells 5 x 384 wells

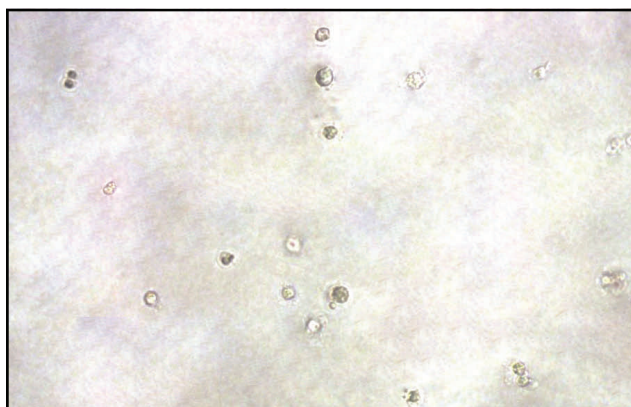
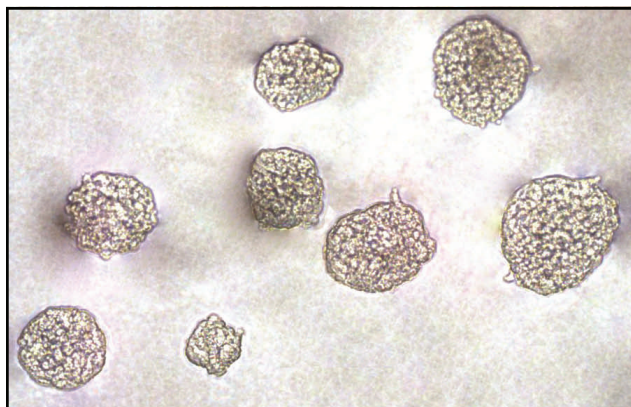
\*Our 96-Well Cell Transformation Assays may be adapted for use with 48-, 24-, 12- or 6-well plates. Kits contain sufficient reagents for 1 plate or 5 plates as noted, regardless of plate format used.

## Easily measure effects of drug compounds on tumor cells in 3-dimensional culture

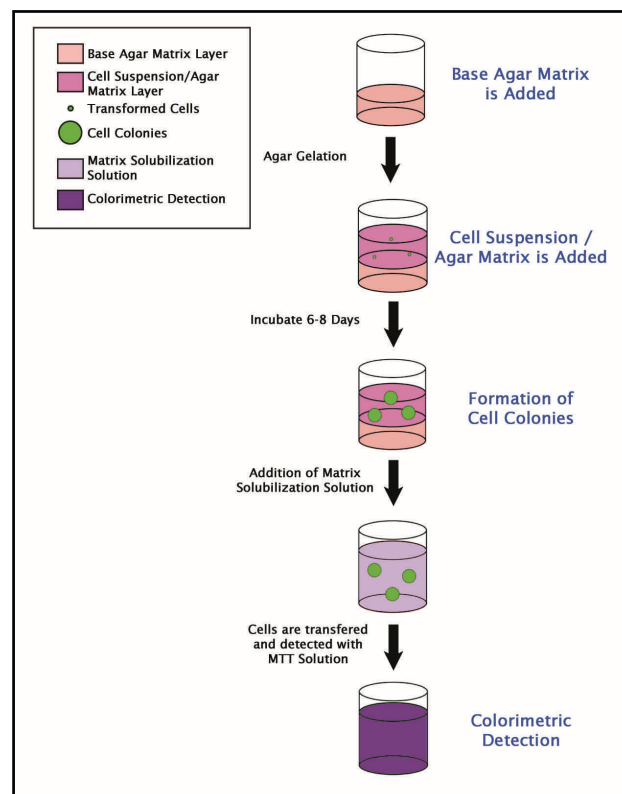
### *In Vitro* Tumor Sensitivity Assay

The CytoSelect™ *In Vitro* Tumor Sensitivity Assay provides a stringent, anchorage-independent model for chemosensitivity testing and possible anticancer drug screening.

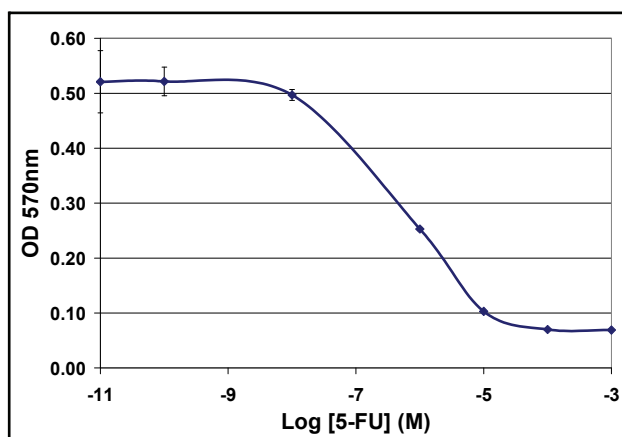
The assay uses a soft agar matrix to promote the colony formation of neoplastic cells in about 6-8 days. The matrix resembles a 3-dimensional cell environment. Treated and untreated cells are then quantified using a standard ELISA plate reader. No manual counting of cells is required.



HeLa Cells Cultured for 7 Days in the Absence (top) or Presence (bottom) of 1  $\mu$ M Taxol using the CytoSelect™ 96-Well In Vitro Tumor Sensitivity Assay.



Assay Principle for the CytoSelect™ 96-Well In Vitro Tumor Sensitivity Assay.



Inhibition of HeLa Cell Transformation by 5-Fluorouracil after a 7-day culture using the CytoSelect™ 96-Well In Vitro Tumor Sensitivity Assay.



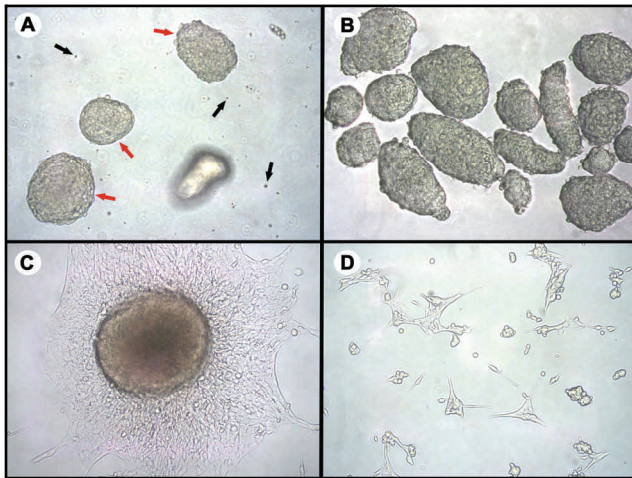
Efficiently isolate clonogenic tumor cells in heterogeneous tissue samples

Clonogenic Tumor Cell Isolation Kit

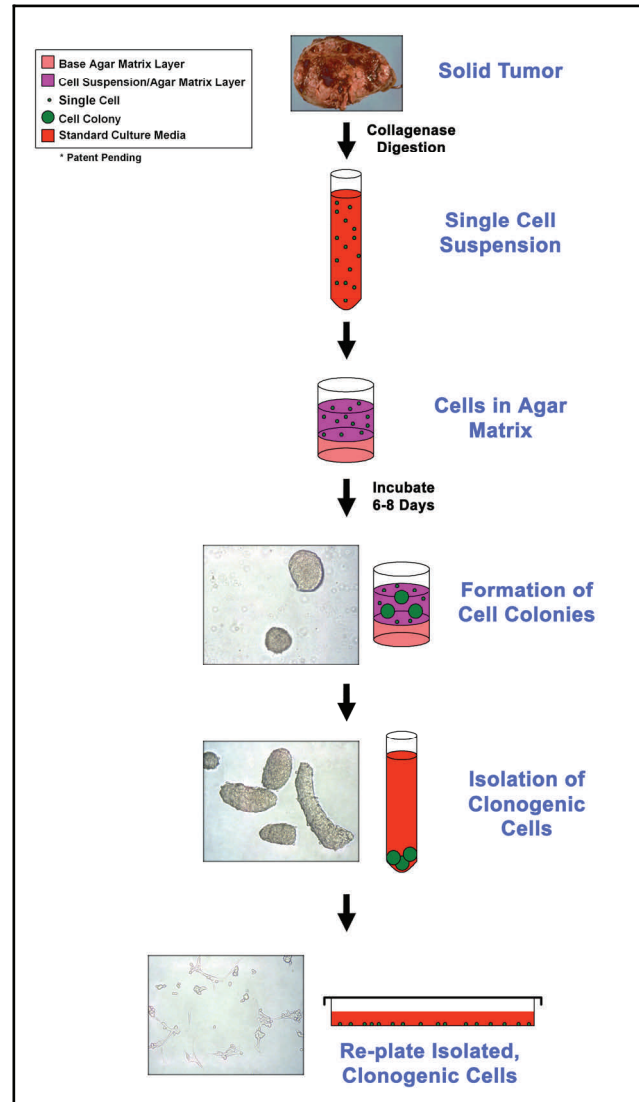
Clean separation of clonogenic tumor cells from normal cells is critical for proper analysis of disease state progression. Due to the heterogeneity of many tumors, however, isolation of homogenous tumor cell populations can be difficult.

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.

In addition to isolation of clonogenic cells from solid tumors, this kit has potential for use in isolating tumor stem cells.



Clonogenic Cell Colony Formation Amidst Normal Cells (A), Clonogenic Cell Isolation (B), and Replating of Isolated Tumor Cells without (C) and with (D) Trypsinization.



Procedure for Isolation of Tumor Cells from a Heterogeneous Tumor Sample using the CytoSelect™ Clonogenic Tumor Cell Isolation Kit.

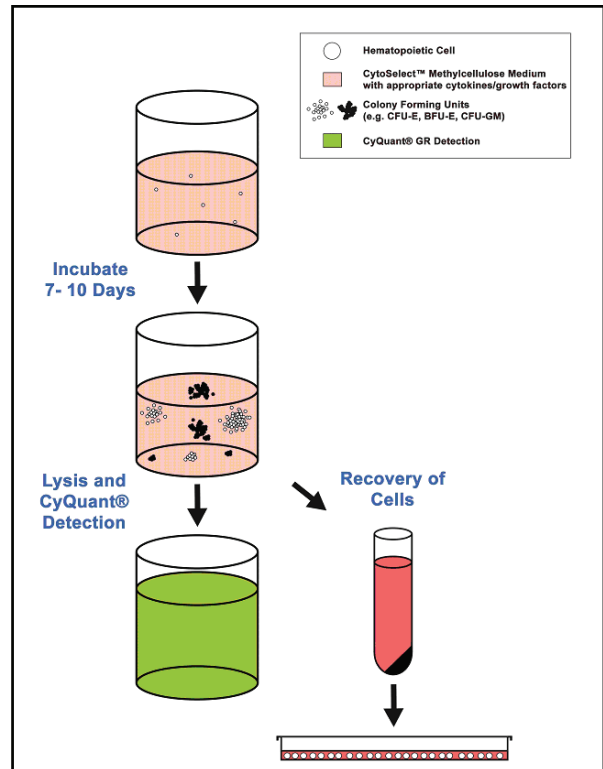
## Quantify colony-forming cells (CFCs) in methylcellulose in a fraction of the time with no manual cell counting

### Hematopoietic Colony Forming Cell Assay

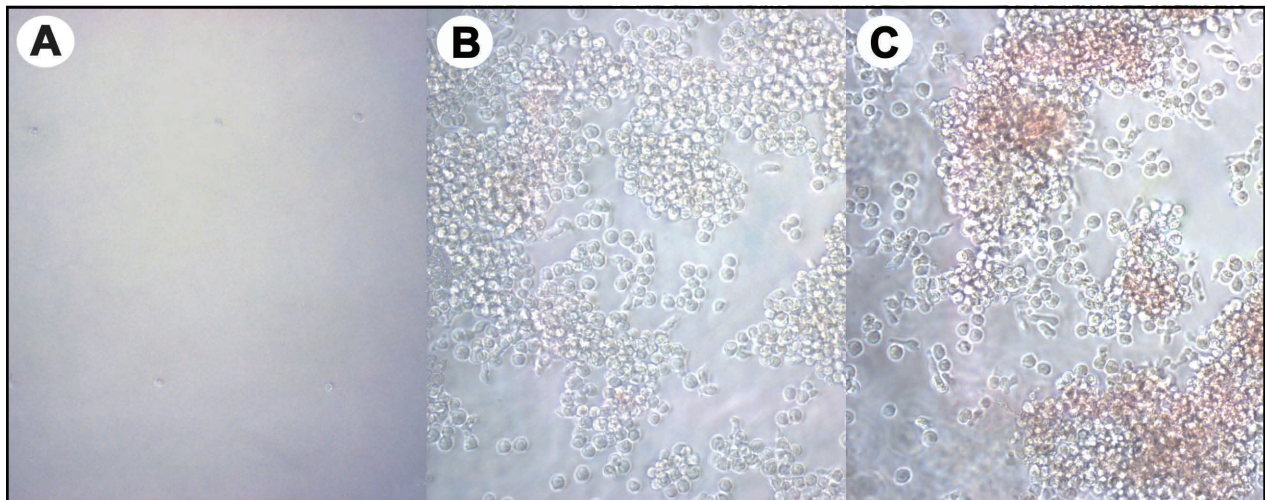
Hematopoietic stem cells (HSCs), when cultured in a suitable semisolid matrix such as methylcellulose supplemented with cytokines & nutrients, proliferate to form discrete cell clusters or colonies. Such HSCs or hematopoietic progenitors are known as colony-forming cells (CFCs).

In classic CFC assays, cells are cultured in a 35mm dish for 14-21 days so the colonies can reach a certain size for manual counting, which can be tedious and subjective.

The CytoSelect™ 96-Well Hematopoietic Colony Forming Cell Assay provides a high-throughput method to quantify CFCs in just 7-10 days with no manual cell counting required. Cells are lysed, solubilized, and quantified using a fluorescent dye included in the kit. Alternatively, cells may be recovered for further culture, analysis, or CFC class determination.



Assay Principle for the CytoSelect™ 96-Well Hematopoietic Colony Forming Cell Assay.



CD34+ Hematopoietic Progenitor Cells from Human Bone Marrow After Culture for 7 Days in the Absence (A) or Presence (B) of Cytokines and After 10 Days in the Presence of Cytokines (C), where Hemoglobin is Visible.

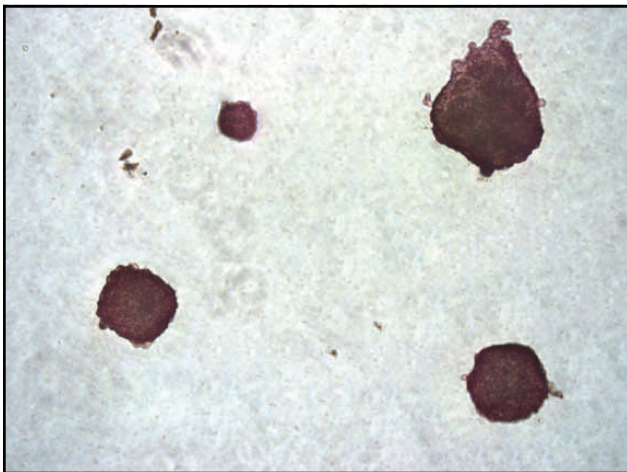
**Quantify embryonic stem cell colonies  
in a high-throughput format in just 7-10 days**

## Stem Cell Colony Formation Assay

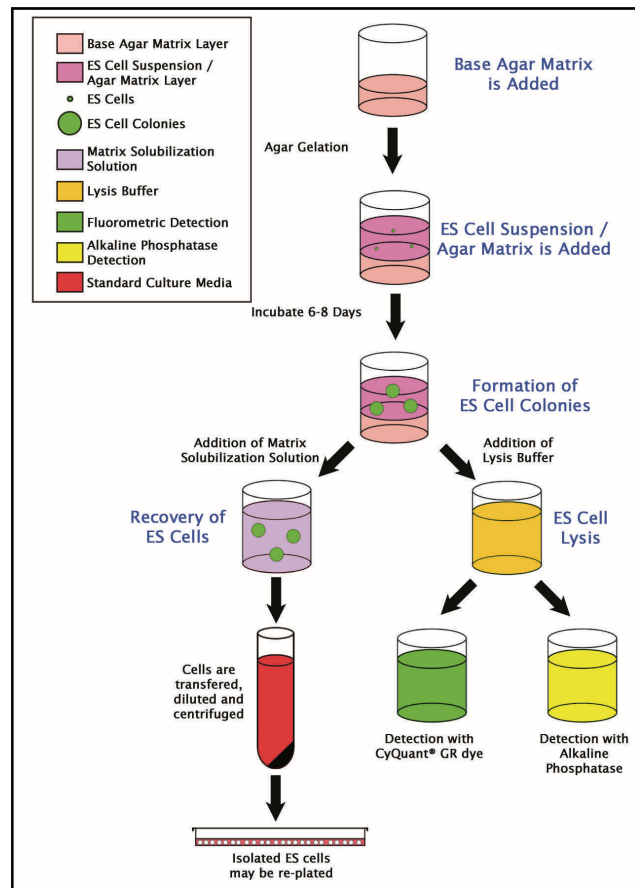
Our StemTAG™ 96-Well Stem Cell Colony Formation Assay provides a high-throughput method to quantify ES cells in just 7-10 days with no manual cell counting required.

After colonies are formed, stem cells may be analyzed in 3 ways:

- Lyse cells, then quantify using a fluorescent dye included in the kit
- Lyse cells, then measure alkaline phosphatase activity using reagents provided
- Recover colonies for further culture and analysis



**Anchorage-Independent Growth of Mouse ES-D3 Cells with Alkaline Phosphatase Staining using the StemTAG™ 96-Well Stem Cell Colony Formation Assay.**



**Assay Principle for the StemTAG™ 96-Well Stem Cell Colony Formation Assay.**

### Trademark Information

CytoSelect and StemTAG are trademarks of Cell Biolabs, Inc.

CyQuant is a registered trademark of Life Technologies Corporation.

## Ordering Information and Published Citations

### Cell Transformation Assays

Product Name	Detection	Size / Qty	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 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.

#### Recent Product Citations

- Gupta, P. et al. (2013). Ocrasin targets the JNK-NFκB axis to sensitize glioma cells to TNF-alpha-induced apoptosis. *Carcinogenesis* **34**:388-396. (CBA-130)
- Xing, C. et al. (2013). Reversing effect of ring finger protein 43 inhibition on malignant phenotypes of human hepatocellular carcinoma. *Mol. Cancer Ther.* **12**:94-103. (CBA-130)
- Rai, V. et al. (2012). Lysophosphatidic acid targets vascular and oncogenic pathways via RAGE signaling. *J. Exp. Med.* **209**:2339-2350. (CBA-130)
- Dennis, M. et al. (2012). Snail controls the mesenchymal phenotype and drives erlotinib resistance in oral epithelial and head and neck squamous cell carcinoma cells. *Carcinogenesis* **147**:726-732. (CBA-130)
- 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)
- 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)
- 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)

### Tumor Sensitivity Assay

Product Name	Detection	Size / Qty	Catalog Number
CytoSelect™ 96-Well <i>In Vitro</i> Tumor Sensitivity Assay	Colorimetric	96 Assays	CBA-150
		5 x 96 Assays	CBA-150-5

#### Recent Product Citations

- 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.
- 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 EGFR T790M mutation. *Cancer Discovery* **2**:922-933.

### Clonogenic Tumor Cell Isolation Kit

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

### Hematopoietic Colony Forming Cell Assay

Product Name	Detection	Size / Qty	Catalog Number
CytoSelect™ 96-Well Hematopoietic Colony Forming Cell Assay	Fluorometric	96 Assays	CBA-320
		5 x 96 Assays	CBA-320-5

### Stem Cell Colony Formation Assay

Product Name	Detection	Size / Qty	Catalog Number
StemTAG™ 96-Well Stem Cell Colony Formation Assay	Fluorometric	96 Assays	CBA-325
		5 x 96 Assays	CBA-325-5