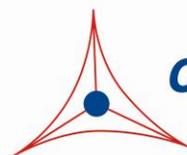

Product Manual

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

Catalog Number

CBA-130	96 assays
CBA-130-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

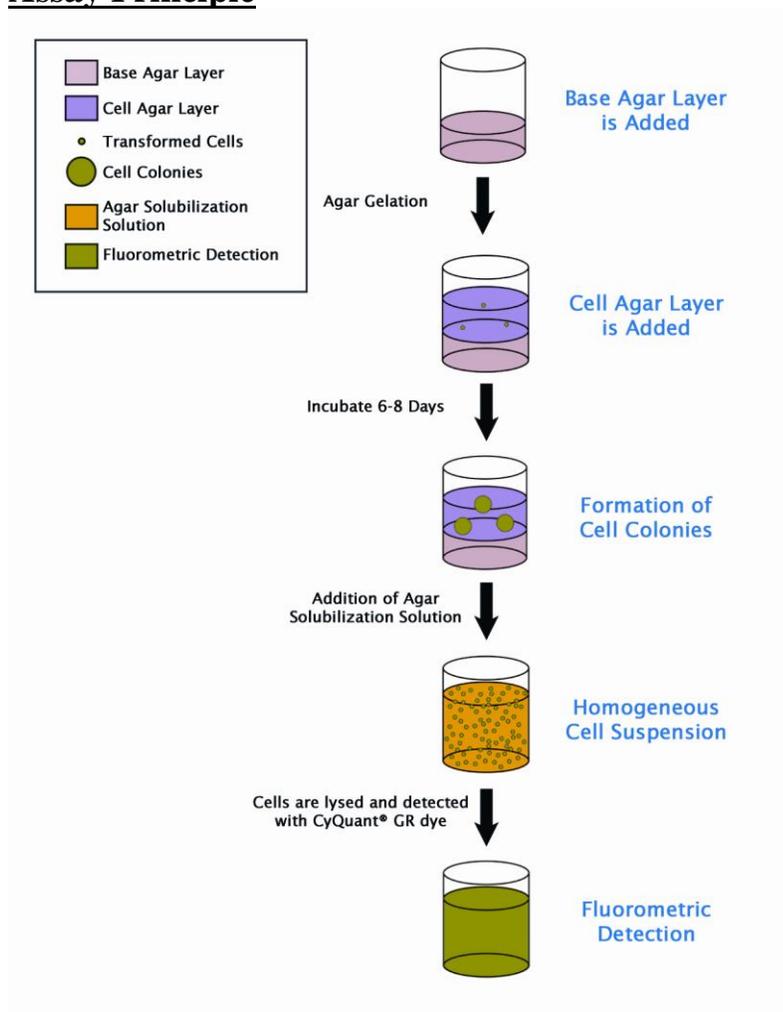
Neoplastic transformation occurs via a series of genetic and epigenetic alterations that yield a cell population that is capable of proliferating independently of both external and internal signals that normally restrain growth. For example, transformed cells show reduced requirements for extracellular growth promoting factors, are not restricted by cell-cell contact, and are often immortal. Anchorage-independent growth is one of the hallmarks of transformation, which is considered the most accurate and stringent in vitro assay for detecting malignant transformation of cells.

Traditionally, the soft agar colony formation assay is a common method to monitor anchorage-independent growth, which measures proliferation in a semisolid culture media after 3-4 weeks by manual counting of colonies. Standard soft agar assays are usually performed in 100-mm or 60 mm dishes, where cells are allowed to grow inside a semisolid culture media for 3-4 weeks before sizable colonies appear. This method is quite cumbersome, time-consuming, and difficult when testing a large number of samples. Additionally, the manual counting of colonies is highly subjective; with varying colony sizes, it's difficult to determine meaningful results.

Cell Biolabs CytoSelect™ 96-well Cell Transformation Assay does **not** involve subjective manual counting of colonies or require a 3–4-week incubation period. Instead, cells are incubated only 6-8 days in a semisolid agar media before being solubilized, lysed and detected by the patented CyQuant® GR Dye in a fluorescence plate reader (see Assay Principle below). This format provides a quantitative, high-throughput method to accurately measure cell transformation. Additionally, the short incubation time (6-8 days) makes it possible to assay cells transiently transfected with oncogenes or siRNA.

The CytoSelect™ 96-well Cell Transformation Kit provides a robust system for screening oncogenes and cell transformation inhibitors. Each kit provides sufficient quantities to perform 96 tests in a microtiter plate.

Assay Principle



Related Products

1. CBA-106-C: CytoSelect™ 96-Well Cell Migration and Invasion Assay (8µm, Fluorometric)
2. CBA-112: CytoSelect™ 96-Well Cell Invasion Assay (Basement Membrane, Fluorometric)
3. CBA-140: CytoSelect™ 96-Well Cell Transformation Assay (Cell Recovery, Fluorometric)
4. CBA-150: CytoSelect™ In Vitro Tumor Sensitivity Assay
5. CBA-320: CytoSelect™ 96-Well Hematopoietic Colony Forming Cell Assay

Kit Components (shipped at room temperature)

1. CytoSelect™ Agar Powder (Part No. 113001): One 1.2 g bottle
2. 5X DMEM Medium (Part No. 113005): One 5 mL bottle
3. Agar Solubilization Solution (Part No. 113003): One 6 mL amber glass bottle
4. 8X Lysis Buffer (Part No. 113004): One 3 mL bottle
5. CyQuant GR Dye (Part No. 10103): One 25 µL tube

Materials Not Supplied

1. Cells and Culture Medium
2. 1X PBS
3. 37°C Incubator, 5% CO₂ Atmosphere
4. Light Microscope
5. 96-well Fluorometer
6. Microwave or Heating Block
7. Water bath
8. (Optional) Positive Control cells such as NIH 3T3 (Ras G12V)

Storage

Store all components at 4°C.

Preparation of Reagents

- 1.2% Agar Solution: Place 1.2 g of Agar Powder in a sterile bottle, add 100 mL of sterile cell culture grade water. Microwave or boil until agar is completely dissolved.
- 2X DMEM/20% FBS Medium: In a sterile tube, dilute the provided 5X DMEM in sterile cell culture grade water to 2X containing 20% FBS. For example, to prepare a 5 mL solution, add 2 mL of 5X DMEM, 1 mL of FBS and 2 mL of sterile cell culture grade water. Sterile filter the 2X media to 0.2 µm.

Note: You may substitute your own medium in place of the DMEM we provide, but ensure that it is at a 2X concentration.

- CyQuant Working Solution: Immediately before use, prepare sufficient amount of the CyQuant Working Solution by diluting the CyQuant GR Dye 1:400 with 1X PBS. For example, add 10 µL to 4 mL of 1X PBS. Use the solution immediately; do not store the CyQuant Working Solution.

Assay Protocol (must be under sterile conditions)

I. Preparation of Base Agar Layer

1. Melt 1.2% Agar Solution in a microwave and cool to 37°C in a water bath.
2. Warm 2X DMEM/20% FBS medium to 37°C in a water bath. Allow at least 30 minutes for the temperature to equilibrate.
3. Mix equal volumes of 1.2% Agar Solution and 2X DMEM/20% FBS medium in a sterile, pre-warmed tube by inverting several times. Immediately transfer 50 µL of the mixture to each well of a 96-well sterile flat-bottom microplate. Gently tap the plate a few times to allow the agar solution to evenly cover the wells.

Notes:

- *Work quickly with the agar solution to avoid gelation. Also, try to avoid adding air bubbles to the well.*

- *To avoid fast and uneven evaporation that leads to aberrant results, we suggest not using the wells on the plate edge, or filling the edge wells with medium to reduce evaporation.*
4. Transfer the plate to 4°C for 30 minutes to allow the base agar layer to solidify.
 5. Prior to adding the Cell Agar Layer (Section II), allow the plate to warm up for 15 minutes at 37°C.

II. Preparation of Cell Agar Layer (samples should be assayed in triplicate)

1. Melt 1.2% Agar Solution in a microwave and cool to 37°C in a water bath.
2. Warm 2X DMEM/20% FBS medium to 37°C in a water bath. Allow at least 30 minutes for the temperature to equilibrate.
3. Harvest and resuspend cells in culture medium at $0.4 - 4 \times 10^5$ cells/mL, keep the cell suspension warm in a 37°C water bath.
4. Mix equal volumes of 1.2% Agar Solution, 2X DMEM/20% FBS media, and cell suspension (1:1:1) in a sterile, pre-warmed tube by inverting several times. Immediately transfer 75 µL of the mixture to each well of the 96-well flat-bottom microplate already containing the solidified base agar layer (25 µL of cell suspension containing 1000-10000 cells/well will be seeded).
Note: Work quickly with the agar solution to avoid gelation, but gently pipette as not to disrupt the base layer integrity. Also, try to avoid adding air bubbles to the well. Always include negative control wells that contain no cells in the cell agar layer.
5. Transfer the plate to 4°C for 15 minutes to allow the cell agar layer to solidify.

III. Quantitation of Anchorage-Independent Growth

1. Add 100 µL of culture medium containing cell growth activator(s) or inhibitor(s) to each well.
2. Incubate the cells for 6-8 days at 37°C and 5% CO₂. Examine the cell colony formation under a light microscope.
3. Remove culture medium by inverting the plate and blotting on paper towel. Gently tap several times.
4. Add 50 µL of Agar Solubilization Solution to each well of the 96-well plate. Incubate for 1 hr at 37°C.
5. Pipette each well 5-10 times to ensure complete agar solubilization.
6. Add 25 µL of 8X Lysis Buffer to each well. Pipette each well 5-10 times to ensure a homogeneous mixture.
7. Incubate the plate at room temperature for 15 minutes.
8. Transfer 10 µL of the mixture to a 96-well plate suitable for fluorescence measurement.
9. Add 90 µL of the CyQuant Working Solution to each well. Incubate 10 minutes at room temperature.
10. Read the plate in a 96-well fluorometer using a 485/520 nm filter set.

Cell Dose Curve (optional)

1. Harvest and resuspend cells in culture medium at $1 - 5 \times 10^6$ cells/mL.
2. Prepare a serial of 2-fold dilution with culture medium, including a medium blank.
3. Transfer 125 μ L of each cell dilution to a microfuge tube. Add 50 μ L of Agar Solubilization Solution and 25 μ L of 8X Lysis Buffer to each tube. Vortex each tube to ensure a homogeneous mixture. Incubate the tubes at room temperature for 15 minutes.
4. Transfer 10 μ L of the mixture to a 96-well plate suitable for fluorescence measurement.
5. Add 90 μ L of the CyQuant Working Solution to each well. Incubate 10 minutes at room temperature.
6. Read the plate in a 96-well fluorometer using a 485/520 nm filter set.

Example of Results

The following figures demonstrate typical results with the CytoSelect™ 96-well Cell Transformation Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

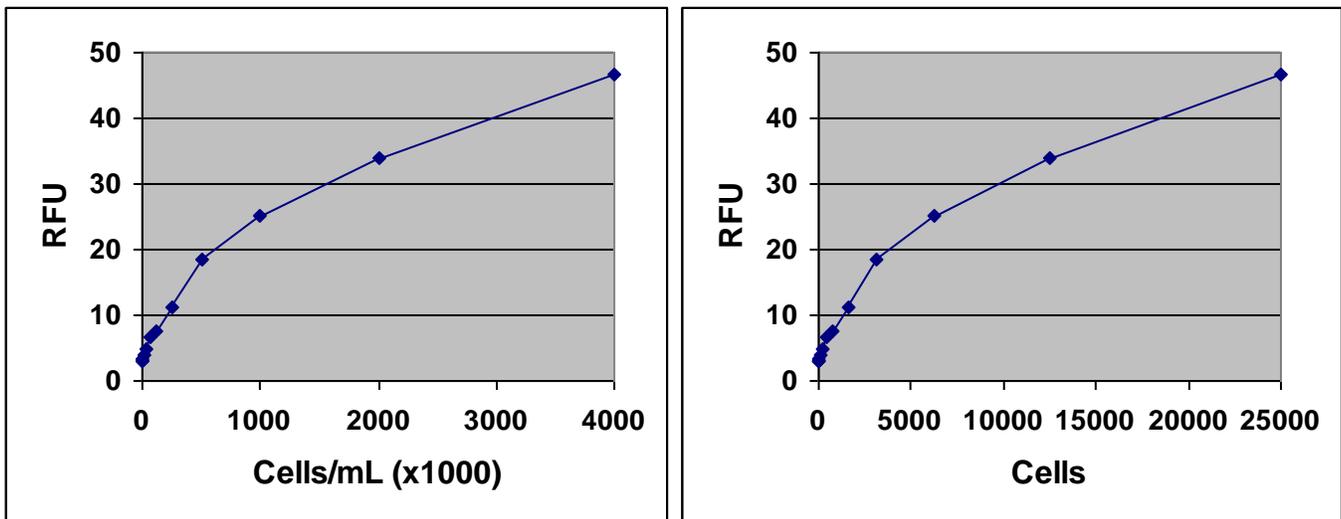


Figure 1. HeLa Cell Dose Curve. Cervical carcinoma HeLa cells were resuspended at 4×10^6 cells/mL and titrated 1:2 in culture medium, followed by addition of Agar Solubilization Solution, Lysis Buffer, and Cyquant® GR Dye detection (as described in the Cell Dose Section). Results were shown by cell concentration or by actual cell number in CyQuant Detection.

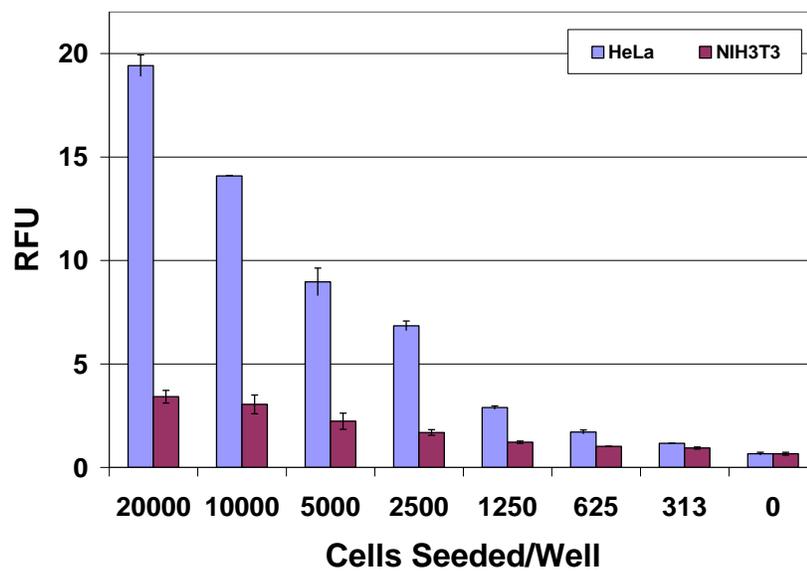


Figure 2. Anchorage-Independent Growth of HeLa Cells. HeLa cells were seeded at various concentrations and cultured for 6 days. HeLa cell transformation is determined according to the assay protocol.

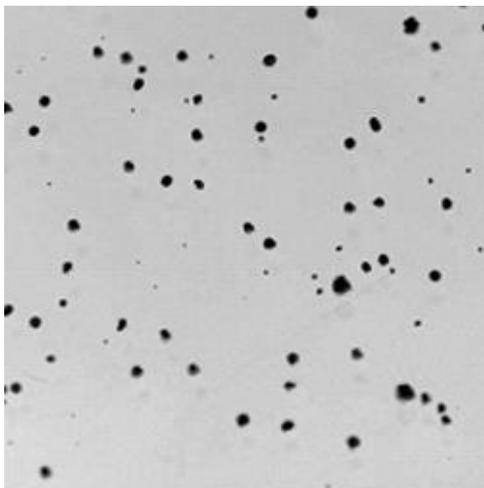


Figure 3. HeLa Colony Formation. HeLa cells were cultured for 14 days according to the assay protocol. Colonies were visualized by 0.1% p-iodonitro tetrazolium violet (INT) staining.

Calculation of Anchorage-Independent Growth

1. Compare RFU values with the Cell Dose Curve and extrapolate the cell concentration in soft agar.
2. Calculate the Total Transformed Cell Number/Well
Total Transformed Cells/Well = cells/mL in soft agar x 0.125 mL/well

For example: If you extrapolate your RFU value from your cell dose curve and determine you have 500,000 cells/mL in your soft agar sample.

Total Transformed Cells/Well = 500,000 cells/mL x 0.125 mL/well = 62,500 cells/well

References

1. Shin SI, Freedman VH, Risser R, and Pollack R. (1975) *Proc Natl Acad Sci U S A*. 72:4435-9.
2. Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW and Weinberg RA. (1999) *Nature* 400:464-8.

Recent Product Citations

1. Choi, B.Y. et al. (2023). Engineered Mesenchymal Stem Cells Over-Expressing BDNF Protect the Brain from Traumatic Brain Injury-Induced Neuronal Death, Neurological Deficits, and Cognitive Impairments. *Pharmaceuticals (Basel)*. **16**(3):436. doi: 10.3390/ph16030436.
2. Ikeda, J. et al. (2023). Hypoxia inducible factor-1 activator munc-18-interacting protein 3 promotes tumour progression in urothelial carcinoma. *Clin Transl Disc*. **3**:e158. doi: 10.1002/ctd2.158.
3. Switzer, C.H. et al. (2022). NOS2 and S-nitrosothiol signaling induces DNA hypomethylation and LINE-1 retrotransposon expression. *Proc Natl Acad Sci U S A*. **119**(21):e2200022119. doi: 10.1073/pnas.2200022119.
4. Furuya, K. et al. (2022). Machine learning extracts oncogenic-specific γ -H2AX foci formation pattern upon genotoxic stress. *Genes Cells*. doi: 10.1111/gtc.13005.
5. Kim, M. et al. (2022). BRAFV600E Mutation Enhances Estrogen-Induced Metastatic Potential of Thyroid Cancer by Regulating the Expression of Estrogen Receptors. *Endocrinol Metab (Seoul)*. **37**(6):879-890. doi: 10.3803/EnM.2022.1563.
6. Toh, P.J.Y. et al. (2022). Optogenetic control of YAP cellular localisation and function. *EMBO Rep*. doi: 10.15252/embr.202154401.
7. Lee, A.R. et al. (2022). Biomarker LEPRE1 induces pelitinib-specific drug responsiveness by regulating ABCG2 expression and tumor transition states in human leukemia and lung cancer. *Sci Rep*. **12**(1):2928. doi: 10.1038/s41598-022-06621-w.
8. Wang, Y. et al. (2022). Long non-coding RNA OIP5-AS1 suppresses microRNA-92a to augment proliferation and metastasis of ovarian cancer cells through upregulating ITGA6. *J Ovarian Res*. **15**(1):25. doi: 10.1186/s13048-021-00937-3.
9. Andriolo, G. et al. (2021). GMP-Grade Methods for Cardiac Progenitor Cells: Cell Bank Production and Quality Control. *Methods Mol Biol*. doi: 10.1007/7651_2020_286.
10. Tan, T.T. et al. (2021). Assessment of Tumorigenic Potential in Mesenchymal-Stem/Stromal-Cell-Derived Small Extracellular Vesicles (MSC-sEV). *Pharmaceuticals*. **14**(4):345. doi: 10.3390/ph14040345.
11. Lo, E.K.K. et al. (2021). Low dose of zearalenone elevated colon cancer cell growth through G protein-coupled estrogenic receptor. *Sci Rep*. **11**(1):7403. doi: 10.1038/s41598-021-86788-w.
12. Park, S. et al. (2021). Cerebral Cavernous Malformation 1 Determines YAP/TAZ Signaling-Dependent Metastatic Hallmarks of Prostate Cancer Cells. *Cancers (Basel)*. **13**(5):1125. doi: 10.3390/cancers13051125.
13. Huang, S.B. et al. (2021). Androgen deprivation-induced elevated nuclear SIRT1 promotes prostate tumor cell survival by reactivation of AR signaling. *Cancer Lett*. doi: 10.1016/j.canlet.2021.02.008.
14. Gao, C. et al. (2020). High intratumoral expression of eIF4A1 promotes epithelial-to-mesenchymal transition and predicts unfavorable prognosis in gastric cancer. *Acta Biochim Biophys Sin (Shanghai)*. pii: gmz168. doi: 10.1093/abbs/gmz168.
15. Eckerdt, F.D. et al. (2020). Combined PI3K α -mTOR Targeting of Glioma Stem Cells. *Sci Rep*. **10**(1):21873. doi: 10.1038/s41598-020-78788-z.

16. Byun, H.J. et al. (2020). LUCAT1 Epigenetically Downregulates the Tumor Suppressor Genes CXXC4 and SFRP2 in Gastric Cancer. *Yonsei Med J.* **61**(11):923-934. doi: 10.3349/ymj.2020.61.11.923.
17. Seo, H.G. et al. (2020). Mutual regulation between OGT and XIAP to control colon cancer cell growth and invasion. *Cell Death Dis.* **11**(9):815. doi: 10.1038/s41419-020-02999-5.
18. Chen, J. et al. (2020). Chrysin serves as a novel inhibitor of DGK α /FAK interaction to suppress the malignancy of esophageal squamous cell carcinoma (ESCC). *Acta Pharm Sin B.* doi: 10.1016/j.apsb.2020.07.011.
19. Inoue, S. et al. (2020). Diffuse mesothelin expression leads to worse prognosis through enhanced cellular proliferation in colorectal cancer. *Oncol Lett.* **19**:1741-1750. doi: 10.3892/ol.2020.11290.
20. Kawai, S. et al. (2020). Three-dimensional culture models mimic colon cancer heterogeneity induced by different microenvironments. *Sci Rep.* **10**(1):3156. doi: 10.1038/s41598-020-60145-9.
21. Kisin, E. R. et al. (2020). Enhanced morphological transformation of human lung epithelial cells by continuous exposure to cellulose nanocrystals. *Chemosphere.* doi: 10.1016/j.chemosphere.2020.126170.
22. Queckbörner, S. et al. (2020). Endometrial stromal cells exhibit a distinct phenotypic and immunomodulatory profile. *Stem Cell Res Ther.* **11**(1):15. doi: 10.1186/s13287-019-1496-2.
23. Song, S. et al. (2019). Cancer Stem Cells of Diffuse Large B Cell Lymphoma Are Not Enriched in the CD45+CD19- cells but in the ALDHhigh Cells. *J. Cancer.* doi: 10.7150/jca.35000.
24. Yang, B. et al. (2019). Stopping transformed cancer cell growth by rigidity sensing. *Nat Mater.* doi: 10.1038/s41563-019-0507-0.
25. Speth, J.M. et al. (2019). Alveolar macrophage secretion of vesicular SOCS3 represents a platform for lung cancer therapeutics. *JCI Insight.* **4**(20). pii: 131340. doi: 10.1172/jci.insight.131340.
26. Kim, D. et al. (2019). Anticancer effect of XAV939 is observed by inhibiting lactose dehydrogenase A in a 3-dimensional culture of colorectal cancer cells. *Oncology Letters.* doi: 10.3892/ol.2019.10813.
27. Copeland, B.T. et al. (2019). Factors that influence the androgen receptor cistrome in benign and malignant prostate cells. *Mol Oncol.* doi: 10.1002/1878-0261.12572.
28. Oliveira-Mateos, C. et al. (2019). The transcribed pseudogene RPSAP52 enhances the oncofetal HMGA2-IGF2BP2-RAS axis through LIN28B-dependent and independent let-7 inhibition. *Nat Commun.* **10**(1):3979. doi: 10.1038/s41467-019-11910-6.
29. Fukuchi, H. et al. (2019). Forkhead box B2 inhibits the malignant characteristics of the pancreatic cancer cell line Panc-1 in vitro. *Genes Cells.* doi: 10.1111/gtc.12717.
30. Salgia, M.M. et al. (2019). Different roles of peroxisome proliferator-activated receptor gamma isoforms in prostate cancer. *Am J Clin Exp Urol.* **7**(3):98-109.

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