

Datasheet for ABIN2344883

CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation)[Go to Product page](#)

70 Publications

Overview

Quantity:	96 tests
Reactivity:	Mammalian
Application:	Cellular Assay (CA)

Product Details

Brand:	CytoSelect™
Sample Type:	Cell Samples
Characteristics:	<p>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 Trial Size kit provides sufficient quantities to perform 24 tests in a 96-well microtiter plate.</p>

Components:	<ol style="list-style-type: none">1. CytoSelect™ Agar Powder : One 0.24 g tube2. 5X DMEM Solution : One 1.5 mL sterile tube3. Agar Solubilization Solution : One 1.5 mL amber tube4. 8X Lysis Buffer : One 1 mL tube5. CyQuant GR Dye : One 10 µL tube
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Material not included:	<ol style="list-style-type: none">1. Cells and Culture Medium2. 1X PBS
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Product Details

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)

Target Details

Background: 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.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	<ul style="list-style-type: none">• Uses traditional 3D soft agar matrix• Fully quantify cell transformation with no manual cell counting• Results in 7-8 days, not 3 weeks
Assay Time:	7 - 8 d
Reagent Preparation:	<ul style="list-style-type: none">• 1.2 % Agar Solution: Place 0.24 g of Agar Powder in a sterile bottle, then add 20 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 2.5 mL solution, add 1 mL of 5X DMEM, 0.5 mL of FBS and 1 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. 4

Assay Procedure:

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 x 10⁵ 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. 5
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

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- 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 2-fold serial dilution with culture medium, including a medium blank.
 3. Transfer 125 µL of each cell dilution to a microfuge tube. Add 50 µL of Agar Solubilization Solution and 25 µL 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 µL of the mixture to a 96-well plate suitable for fluorescence measurement.
 5. Add 90 µL 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. 6

Calculation of Results:

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

Restrictions:

For Research Use only

Handling

Storage:

4 °C

Storage Comment:

Store all components at 4°C.

Publications

Product cited in:

Thompson, MacPherson, Beuschel, Shukla: "Asbestos-Induced Mesothelial to Fibroblastic Transition Is Modulated by the Inflammasome." in: **The American journal of pathology**, Vol. 187, Issue 3, pp. 665-678, (2017) ([PubMed](#)).

Otaka, Rokudai, Kaira, Fujieda, Horikoshi, Iwakawa-Kawabata, Yoshiyama, Yokobori, Ohtaki, Shimizu, Oyama, Tamura, Prives, Nishiyama: "STXBP4 Drives Tumor Growth and Is Associated

with Poor Prognosis through PDGF Receptor Signaling in Lung Squamous Cell Carcinoma." in:

Clinical cancer research : an official journal of the American Association for Cancer Research, (2017) ([PubMed](#)).

Illuzzi, McNeill, Bastian, Brenerman, Wersto, Russell, Bunz, McKinnon, Becker, Wilson: "Tumor-associated APE1 variant exhibits reduced complementation efficiency but does not promote cancer cell phenotypes." in: **Environmental and molecular mutagenesis**, Vol. 58, Issue 2, pp. 84-98, (2017) ([PubMed](#)).

Wang, Cui, Xu, Irudayaraj: "Basic studies on epigenetic carcinogenesis of low-dose exposure to 1-trichloromethyl-1,2,3,4-tetrahydro- β -carboline (TaClo) in vitro." in: **PLoS ONE**, Vol. 12, Issue 2, pp. e0172243, (2017) ([PubMed](#)).

Manzanares, Usui, Campbell, Dumur, Maldonado, Fausther, Dranoff, Sirica: "Transforming Growth Factors α and β Are Essential for Modeling Cholangiocarcinoma Desmoplasia and Progression in a Three-Dimensional Organotypic Culture Model." in: **The American journal of pathology**, Vol. 187, Issue 5, pp. 1068-1092, (2017) ([PubMed](#)).

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