

Datasheet for ABIN2344883 CytoSelect[™] 96-Well Cell Transformation Assay (Soft Agar **Colony Formation**)

70

Publications



Overview

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

Product Details

Brand:	CytoSelect™
Sample Type:	Cell Samples
Characteristics:	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 $^{\ensuremath{ extsf{B}}}$
	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.
Components:	1. CytoSelect™ Agar Powder : One 0.24 g tube
	2. 5X DMEM Solution : One 1.5 mL sterile tube
	3. Agar Solubilization Solution : One 1.5 mL amber tube
	4. 8X Lysis Buffer : One 1 mL tube
	5. CyQuant GR Dye : One 10 µL tube
Material not included:	1. Cells and Culture Medium
	2. 1X PBS

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- 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:	 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:	 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

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	 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
	 Melt 1.2 % Agar Solution in a microwave and cool to 37 °C in a water bath. Warm 2X DMEM/20 % FBS medium to 37 °C in a water bath. Allow at least 30 minutes for the temperature to equilibrate. Mix equal volumes of 1.2 % Agar Solution and 2X DMEM/20 % FBS medium in a sterile, prewarmed 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. Transfer the plate to 4 °C for 30 minutes to allow the base agar layer to solidify. 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)
	 Melt 1.2 % Agar Solution in a microwave and cool to 37 °C in a water bath. Warm 2X DMEM/20 % FBS medium to 37 °C in a water bath. Allow at least 30 minutes for the temperature to equilibrate. 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. 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. Transfer the plate to 4 °C for 15 minutes to allow the cell agar layer to solidify.
	III. Quantitation of Anchorage-Independent Growth
	 Add 100 μL of culture medium containing cell growth activator(s) or inhibitor(s) to each well. 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 μ L 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 μ L 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 $1 - 5 \times 106$ cells/ml
	 Prepare a 2-fold serial dilution with culture medium including a medium blank
	3. Transfer 125 ul. of each cell dilution to a microfuge tube. Add 50 ul. of Agar Solubilization
	Solution and 25 µL of 8X Lysis Buffer to each tube. Vortex each tube to ensure a
	4 Transfer 10 ul. 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.
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Publications	
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