Datasheet for ABIN2344889

CytoSelect™ 96-Well Cell Transformation Assay (Cell Recovery Compatible, Fluorometric)

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Overview

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

Product Details

Brand:	CytoSelect™
Sample Type:	Cell Samples
Detection Method:	Fluorometric
Characteristics:	CytoSelect™ 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 proprietary semisolid agar media before being solubilized, lysed and detected by the patented CyQuant® GR Dye in a fluorescence plate reader (see Assay Principle below). Alternatively, viable transformed cells can be easily recovered for further culturing and testing, such as in protein/DNA array analysis and cancer vaccine development. This format provides a quantitative, high-throughput method to accurately measure cell transformation, while the short incubation time makes it possible to assay cells transiently transfected with oncogenes or siRNA. The CytoSelect™ Cell Transformation Kit (Cell Recovery Compatible) provides a robust system for measuring in vitro drug sensitivity, screening oncogenes and cell transformation inhibitors, and allows for transformed cell recovery. Each Trial Size kit provides sufficient quantities to perform 24 assays in a 96-well plate, 12 assays in a 48-well plate, 6 assays in a 24-well plate, or 3 assays in a 12-well plate.
Components:	1. 10X CytoSelect™ Agar Matrix Solution : One 1 mL sterile tube

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	2. CytoSelect™ Matrix Diluent : One 1 mL sterile tube 3. 5X DMEM Solution : One 1.5 mL sterile tube 4. 10X Matrix Solubilization Solution : One 0.6 mL sterile tube 5. 4X Lysis Buffer : One 2 mL tube 6. CyQuant® GR Dye : One 25 µL tube
Material not included:	1. Cells and Culture Medium 2. 37 °C Incubator, 5 % CO2 Atmosphere 3. Light Microscope 4. 96-well Fluorometer 5. 37 °C and boiling water baths 6. (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:	• Proprietary modified soft agar medium • Fully quantify cell transformation with no manual cell counting • Results in 7-8 days, not 3 weeks • Recover cells from soft agar medium for further downstream analysis
Assay Time:	7 - 8 d

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Reagent Preparation:

- 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.
- 1X Matrix Solubilization Solution: Prepare a 1X Matrix Solubilization Solution by diluting the provided 10X stock 1:10 in sterile cell culture grade water. Sterile filter the 1X solution to 0.2 µ m.
- 10X CytoSelect™ Agar Matrix Solution: Heat the Agar Matrix Solution tube to 90-95 °C in a water bath for 30 minutes, or until agar matrix liquefies. Transfer the tube to a 37 °C water bath for 20 minutes and maintain until needed. 4

Assay Procedure:

The following assay protocol is written for a 96-well format. Refer to the below table for the appropriate dispensing volumes of other plate formats. Culture Dish 96-well 48-well 24-well 12-well 6-well Base Agar Matrix Layer 50 100 250 500 1000 (µL/well) Cell Suspension/Agar 75 150 375 750 1500 Matrix Layer (µL/well) Culture Media (µL/well) 50 100 250 500 1000 1X Matrix Solubilization Solution 125 250 625 1250 2500 (µL/well) Table

1. Dispensing Volumes of Different Plate Formats

I. Preparation of Base Agar Matrix Layer

1. Heat the 10X CytoSelect™ Agar Matrix Solution to 90-95 °C in a water bath for 30 minutes, or until agar matrix liquefies. Transfer the tube to a 37 °C water bath for 20 minutes and maintain until needed.
2. Warm the 2X DMEM/20 % FBS medium (see Preparation of Reagents section) to 37 °C in a water bath. Allow at least 30 minutes for the temperature to equilibrate.
3. According to Table 2 (below), prepare the desired volume of Base Agar Matrix Layer in the following sequence: a. In a sterile tube, add the appropriate volume of 2X DMEM/20 % FBS medium. b. Next, add the corresponding volume of sterile water. Mix well. c. Finally, add the corresponding volume of 10X CytoSelect™ Agar Matrix Solution. Mix well. Note: The 10X CytoSelect™ Agar Matrix Solution is slightly viscous, care should be taken in accurately pipetting the appropriate volume. 2X DMEM/20 % Sterile Water 10X Total Volume of # of Tests in 96- FBS Medium (mL) CytoSelect™ Base Agar Matrix well Plate (50 (mL) Agar Matrix Layer (mL) µL/test) Solution (mL) 0.625 0.5 0.125 1.25 25 Table
4. Preparation of Base Agar Matrix Layer
5. After mixing, maintain the Base Agar Matrix Layer at 37 °C to avoid gelation. 5
6. Dispense 50 µL of Base Agar Matrix Layer into each well of a 96-well sterile flat-bottom microplate (samples should be assayed in triplicate). Gently tap the plate a few times to ensure the Base Agar Matrix Layer evenly covers the wells. Notes: • Work quickly with the layer 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.
7. Transfer the plate to 4 °C for 30 minutes to allow the Base Agar Matrix Layer to solidify.
8. Prior to adding the Cell Suspension/Agar Matrix Layer (Section II), allow the plate to warm

to room temperature for 30 minutes.

II. Addition of Cell Suspension/Agar Matrix Layer (under sterile conditions)

1. Heat the 10X CytoSelect™ Agar Matrix Solution to 90-95 °C in a water bath for 30 minutes, or until agar matrix liquefies. Transfer the tube to a 37 °C water bath for 20 minutes and maintain until needed.
2. Warm the 2X DMEM/20 % FBS medium (see Preparation of Reagents section) and CytoSelect™ Matrix Diluent 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 0.1 - 1 x 106 cells/mL. Keep the cell suspension warm in a 37 °C water bath.
4. According to Table 3 (below), prepare the desired volume of Cell Suspension/Agar Matrix Layer in the following sequence: a. In a sterile tube, add the appropriate volume of 2X DMEM/20 % FBS medium. b. Next, add the corresponding volume of CytoSelect™ Matrix Diluent. Mix well. c. Next, add the corresponding volume of 10X CytoSelect™ Agar Matrix Solution. Mix well. d. Finally, add the corresponding volume of cell suspension. Mix well. Note: The CytoSelect™ Matrix Diluent and 10X CytoSelect™ Agar Matrix Solution are slightly viscous, care should be taken in accurately pipetting the appropriate volumes. 2X CytoSelect™ 10X Cell Total Volume of # of Tests in DMEM/20 % Matrix Diluent CytoSelect™ Suspension Cell Suspension/ 96-well Plate FBS Medium (mL) Agar Matrix (mL) Agar Matrix (75 µL/test) (mL) Solution (mL) Layer (mL) 0.875 0.688 0.188 0.125 1.875 25 Table
5. Preparation of Cell Suspension/Agar Matrix Layer 6
6. After mixing, incubate the Cell Suspension/Agar Matrix Layer at room temperature for 5 minutes.
7. Immediately dispense 75 µL of Cell Suspension/Agar Matrix Layer into each well of the 96-well plate, already containing the Base Agar Matrix Layer (Section I). Notes: • Work quickly with the layer 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 Suspension/Agar Matrix Layer.
8. Transfer the plate to 4 °C for 20 minutes to allow the Cell Suspension/Agar Matrix Layer to solidify.
9. Allow the plate to warm to room temperature for 30 minutes.
10. Add 50 µL of culture medium containing cell growth activator(s) or inhibitor(s) to each well.
11. Incubate the cells for 6-8 days at 37 °C and 5 % CO2. Examine the colony formation under a light microscope.

III. Quantitation of Anchorage-Independent Growth (skip to section IV if cell recovery/re-plating is desired)

1. Add 125 µL of 1X Matrix Solubilization Solution to each well.
2. Pipette the entire volume of the well 10-12 times to mix thoroughly and solubilize the agar matrix completely.
3. Transfer 150 µL of the mixture to a 96-well plate suitable for fluorescence measurement.
4. Prepare sufficient 4X Lysis Buffer/CyQuant® GR dye solution for all samples by diluting the dye 1:75 in 4X Lysis Buffer (for example, add 5 µL dye to 370 µL of 4X Lysis Buffer).
5. Add 50 µL of 4X Lysis Buffer/CyQuant® GR dye solution to each well (already containing

150 µL of solution). Incubate the plate at room temperature for 30 minutes.
6. Pipette each well 7-10 times to ensure a homogeneous mixture.
7. Read the plate in a 96-well fluorometer using a 485/520 nm filter set.

IV. Cell Recovery and Re-plating (under sterile conditions)

1. Add 125 µL of 1X Matrix Solubilization Solution to each well.
2. Pipette each well 10-12 times to mix thoroughly.
3. Transfer the entire mixture to at least 20 volumes of standard culture medium (for example, 1 mL would be transferred to 20 mL media).
4. Pipette the mixture vigorously 7-10 times.
5. Centrifuge the cell pellet and aspirate the media supernatant. 7
6. Resuspend the cell pellet in another 20 volumes of standard culture medium.
7. Repeat steps 4-6.
8. Resuspend the pellet and transfer to a tissue culture flask or dish.
9. Transfer to a cell culture incubator. Cell Dose Curve (optional)
    1. Heat the 10X CytoSelect™ Agar Matrix Solution to 90-95 °C in a water bath for 30 minutes, or until agar matrix liquefies. Transfer the tube to a 37 °C water bath for 20 minutes and maintain until needed.
    2. Warm the 2X DMEM/20 % FBS medium (see Preparation of Reagents section) and CytoSelect™ Matrix Diluent 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 1 - 5 x 106 cells/mL.
    4. Prepare a serial 2-fold dilution in culture medium, including a blank without cells.
    5. Transfer 50 µL of each dilution to a 96-well plate.
    6. According to Table 4 (below), prepare the desired volume of Cell Dose Curve Solution in the following sequence: a. In a sterile tube, add the appropriate volume of 2X DMEM/20 % FBS medium. b. Next, add the corresponding volume of sterile water. Mix well. c. Next, add the corresponding volume of CytoSelect™ Matrix Diluent. Mix well. d. Finally, add the corresponding volume of 10X CytoSelect™ Agar Matrix Solution. Mix well. Note: The CytoSelect™ Matrix Diluent and 10X CytoSelect™ Agar Matrix Solution are slightly viscous, care should be taken in accurately pipetting the appropriate volumes. 2X DMEM/20 % Sterile Water CytoSelect™ 10X CytoSelect™ Total Volume of FBS Medium (mL) Matrix Diluent Agar Matrix Cell Dose Curve (mL) (mL) Solution (mL) Solution (mL) 1.25 0.45 0.55 0.25 2.5 0.625 0.225 0.275 0.125 1.25 Table
    7. Preparation of Cell Dose Curve Solution
    8. Immediately dispense 125 µL of Cell Dose Curve Solution into the wells of the 96-well plate, already containing the cell serial dilution (from step 5).
    9. Add 125 µL of 1X Matrix Solubilization Solution to each well. Pipette each well 10-12 times to mix thoroughly.
    10. Transfer 150 µL of the mixture to a 96-well plate suitable for fluorescence measurement.
    11. Prepare sufficient 4X Lysis Buffer/CyQuant® GR dye solution for all samples by diluting the dye 1:75 in 4X Lysis Buffer (for example, add 5 µL dye to 370 µL of 4X Lysis Buffer). 8
    12. Add 50 µL of 4X Lysis Buffer/CyQuant® GR dye solution to each well (already containing

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	150 µL of solution). Incubate the plate at room temperature for 30 minutes. 13. Pipette each well 7-10 times to ensure a homogeneous mixture. 14. Read the plate in a 96-well fluorometer using a 485/520 nm filter set.
Calculation of Results:	1. Compare RFU values with the Cell Dose Curve and extrapolate the cell concentration. 2. Calculate the Total Transformed Cell Number/Well Total Transformed Cells/Well = cells/mL x 0.050 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 sample. Total Transformed Cells/Well = 500,000 cells/mL x 0.050 mL/well = 25,000 cells/well
Restrictions:	For Research Use only

Handling

Storage:	4 °C
Storage Comment:	Store all components at 4°C.

Publications

Product cited in:

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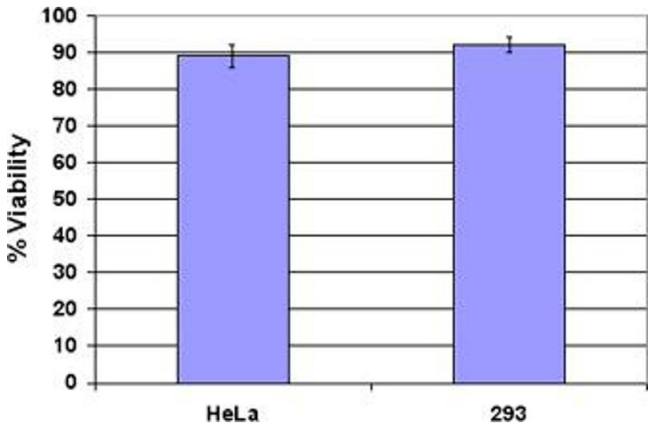
Muruganandan, Parlee, Rourke, Ernst, Goralski, Sinal: "Chemerin, a novel peroxisome proliferator-activated receptor gamma (PPARgamma) target gene that promotes mesenchymal

stem cell adipogenesis." in: **The Journal of biological chemistry**, Vol. 286, Issue 27, pp. 23982-95, (2011) (PubMed).

Images



Cellular Assay

Image 1. Viability of Recovered Cells. HeLa and 293 cells were cultured for 6 days according to the assay protocol. Cells were recovered and the cell viability was determined by trypan blue exclusion.