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ab235699 Cell Transformation Assay Kit (Fluorometric)

For the measurement of cell transformation in mammalian adherent or suspension cells in response to stimuli that inhibit or induce transformation.

This product is for research use only and is not intended for diagnostic use.

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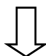
1. Overview

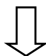
Transformed cells can proliferate without attaching to a surface. Anchorage-independent cell growth is the hallmark of cell transformation. The Soft-Agar Assay is a traditional method for screening cell transformation in vitro. However, this method is lengthy (3-4 weeks incubation), laborious (counting colonies) and inconsistent (due to subjective counting). The Cell Transformation Assay Kit (Fluorometric) (ab235699) is faster, stable and more sensitive than the traditional Soft-Agar Assay. The kit uses a quantitative dye that binds to nucleic acid and generates green fluorescence. This one-step method is non-radioactive and simple (just add-and-read, and does not require tedious labor such as counting colonies). The assay is high-throughput adaptable and has wide linear range from 50-60,000 cells. The entire assay can be finished within 7-8 days.

Prepare cell-dose curve and time zero samples.

Measure fluorescence at Ex/Em = 480/530 nm at 450 nm

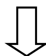


Prepare Base Agarose Layer

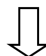


Prepare Top Agarose Layer containing blank and cells of interest +/- test compounds.

Incubate for 6-8 days at 37°C



Remove media and add Agarose Solubilizing Solution.



Transfer to 96-well white plate. Add 1X Quantitative Dye Solution and measure fluorescence at Ex/Em = 480/530 nm

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Agarose Powder	240 mg	-20°C	-20°C
DMEM Solution (10X)	2 x 1.5 mL	-20°C	-20°C
Staining Solution	1 mL	-20°C	-20°C
Agarose Solubilization Solution	5 mL	-20°C	-20°C
Quantitative Dye (200X)	0.1 mL	-20°C	Do not store

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well clear tissue culture plate and 96-well white plate
- Sterile dH_2O, PBS, and FBS
- Microscope
- Multi-well spectrophotometer (ELISA reader) capable of making measurements at Ex/Em = 480/530 nm

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

ΔNote: Prepare reagents and perform assays under sterile conditions (i.e. tissue culture hood/biosafety cabinet).

5.1 Agarose Powder

1. To make 1.2% agarose solution, add 20 mL of sterile dH_2O into the Agarose Powder bottle.
2. Open the bottle cap slightly, and heat the bottle on a heat block until the Agarose Powder is completely dissolved (~100°C; 30-40 minutes is recommended). Gently shake the bottle to solubilize the agarose.
3. Transfer the bottle to a 37°C water bath and keep it for 30 minutes to equilibrate temperature.
4. Unused 1.2% agarose solution can be stored at 4°C under sterile conditions.

ΔNote: Keep the Agarose solution in a 37 °C water bath throughout cell-seeding process to prevent solidification of the agarose solution.

5.2 DMEM Solution (10X)

1. Dilute 10X DMEM in sterile dH_2O to 1X DMEM containing 10% FBS (1X DMEM/10% FBS). For example, dilute 100 µL of DMEM Solution (10X) into 900 µL dH_2O with 100 µL of FBS.
2. Make as much as needed. Store at 4°C. Before using, warm to 37°C in a water bath.

5.3 Quantitive Dye (200X)

1. To make 1X Quantitative Dye Solution, dilute 200X Quantitative Dye with 1X PBS. For example: add 10 µL of 200X Quantitative Dye into 1.99 mL 1X PBS, mix well.
2. Discard the unused 1X Quantitative Dye Solution and always prepare fresh dilution.

5.4 Staining Solution

Ready to use as supplied.

5.5 Agarose Solubilization Solution

Ready to use as supplied.

6. Assay Procedure

– Assay all standards, controls and samples in duplicate.

6.1 Preparation of Base Agarose layer:

1. Prepare 75 µL/well base agarose mix as follows:

Component	Volume (µL)
1.2% Agarose solution	37.5
DMEM Solution (10X)	7.5
FBS	7.5
dH_2O	22.5

2. Prepare enough Base Agarose mix for the number of experiments to be performed. Mix well.
3. Add 75 µL of base agarose mix into desired wells in a 96-well clear bottom tissue culture plate. Keep the plate at 4°C for 15 minutes to solidify the agarose.

ΔNote: Prior to adding the top layer with cells, warm the plate at room temperature by keeping in a tissue culture hood for 10 minutes.

6.2 Preparation of Top Agarose Layer with cells:

1. Prepare a stock solution of cells (1-5 X 10^6 cells/mL) in 1X DMEM/10% FBS medium.
2. Calculate and adjust the desired concentration (see **ΔNote**, below) based on the number of cells per well per assay.
3. Prepare 75 µL/well of Top Agarose Layer mix as follows:

Component	Volume (µL)
1.2% Agarose solution	25.0
DMEM Solution (10X)	5.5
FBS	5.5
Cells in 1XDMEM/10% FBS	20
dH_2O	19

4. Make as much as needed for the number of sample and cell-dose curve wells. Mix by pipetting.
5. Add 75 µL of agarose-cell mix into each well of a 96-well clear bottom tissue culture plate already containing the solidified base agarose layer. Keep the plate at 4°C for 10 minutes to solidify the top agarose-cell mix.
6. Bring the plate to room temperature by keeping it in the tissue culture hood for 10 minutes.
7. Add a total of 100 µL of 1X DMEM/10% FBS medium with or without test compound into each well and incubate at 37°C for 6-8 days.

ΔNote: Assay has linear range from 50 to 60,000 cells, depending on the cell type used in the experiment. Adjust the cell numbers to avoid over-seeding.

ΔNote: Prepare parallel well(s) as blank control (no cells) with the same amount of culture medium and reagents for the reagent background reading.

ΔNote: During the process of plating the base agarose layer and the top agarose layer, keep 1.2% agarose solution, DMEM solution (10X), sterile dH_2O, and FBS in a 37°C water bath to equilibrate the temperature and to prevent solidification of agarose in case of 1.2% agarose layers.

ΔNote: A multi-channel pipette can be used for plating base agarose layer. Add agarose-cell mix carefully to avoid bubbles in both base and top agarose layers.

ΔNote: Colony Visualization (Optional): Add 10 µL Staining Solution into each well and incubate for 60 minutes at 37°C in an incubator with 5% CO_2. Colonies formed by transformed cells can be visualized and imaged under a microscope.

6.3 Cell-dose curve:

1. On day 0, prepare a cell-dose curve by using the stock made in step 6.2.1 (1-5 X 10^6 cells/ml in 1 X DMEM/10% FBS medium).
2. Prepare eight serial dilutions (2-fold) in separate 1.5 mL centrifuge tubes with 1X DMEM/10% FBS medium (150 µL). Transfer 150 µL of each mixture into a separate well of a 96-well clear plate.
3. Add 50 µL of Agarose Solubilization Solution to each tube, mix and incubate cells for 15 minutes at room temperature.
4. Transfer 20 µL of each mixture into 96- well white plate. Add 80 µL 1X Quantitative Dye Solution to each well, protect from light and shake for 10 minutes on a shaker.
5. Measure fluorescence using a microtiter plate reader at Ex/Em = 430/530 nm.

6.4 Measurement:

1. On day 6-8 (at the end of the desired incubation time, step 6.2), carefully remove the medium on top of the top agarose layer by pipetting.
2. Add 50 µL of Agarose Solubilization Solution into each well and incubate at 37 □C incubator for 1 hour to solubilize the agarose.
3. Transfer 20 µL of Solubilized Agarose-cell mix into a 96-well white plate and add 80 µL of 1X Quantitative Dye Solution. Protect from light and gently shake for 15 minutes at room temperature.
4. Measure fluorescence at Ex/Em = 480/530 nm.

7. Data Analysis

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (0 cells) from all readings. This is the corrected absorbance.
3. Plot the cell-dose curve. Draw the best straight line through these points to construct the cell-dose curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your cell-dose curve data (use the equation that provides the most accurate fit).
4. Apply the sample readings (ΔRFU) to the Cell-Dose curve (cell number/well: 1/10th of the original stock solutions, 6.3) to get the number of transformed cells.

8. FAQs / Troubleshooting

General troubleshooting points are found at
www.abcam.com/assaykitguidelines

9. Typical Data

Data provided **for demonstration purposes only**.

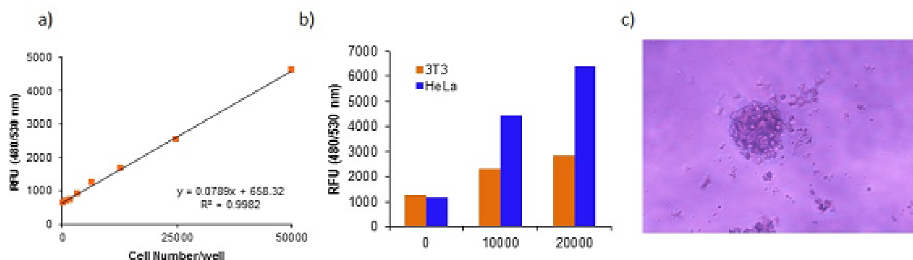


Figure 1. (a) HeLa (Human epithelial cell line from cervix adenocarcinoma) cell-dose curve; (b) Equal numbers of NIH/3T3 (Mouse embryo fibroblast cell line) and HeLa cells were serially diluted and seeded in agarose gel. Cells were solubilized and detected with Quantitative Dye; (c) Image of HeLa cell colonies. HeLa cells were cultured for 7 days according to the kit protocol.

10. Notes

Technical Support

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