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ab235698 Cell Transformation Assay Kit (Colorimetric)

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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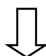
1. Overview

Transformation of normal cells into neoplastic (malignant) cells is the first step in tumorigenesis. In vitro assays of cellular oncotransformation, which measures phenotypic alterations to cultured cells that mirror those observed in tumors, are a critical tool in studying the mechanisms of carcinogenesis. Cell transformation assays are frequently employed in toxicology to evaluate the carcinogenic potential of a particular compound. The Soft-Agar Assay is a traditional method for screening cell transformation *in vitro*. In this assay, transformed cultured cells gain the ability to proliferate through the soft agarose gel without attaching to a surface and tend to form spheroid-like clumps of cells colonies. However, this method is lengthy (3-4 weeks incubation), laborious (counting colonies) and inconsistent (due to subjective counting). Cell Transformation Assay Kit (Colorimetric)(ab235698) is faster, stable and more sensitive than the traditional Soft-Agar Assay. The kit is based on the conversion of the tetrazolium salt (WST) to formazan by cellular mitochondrial dehydrogenases. The generated signal is directly proportional to the number of living cells. This one-step method is non-radioactive and simple (just add-and-read, does not require harvesting cells, and solubilization steps). The assay is high-throughput adaptable and has a wide linear range from 10,000-400,000 cells. The entire Cell Transformation Assay can be finished within 7-8 days.

Prepare cell-dose curve and time zero samples.
Measure absorbance at 450 nm.

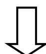


Prepare Base Agarose Layer

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

Incubate for 6-8 days at 37°C

Remove media and add DMEM/FBS + WST Working Solution and incubate for 4 hours at 37°C



Measure absorbance at 450 nm. Optionally, stain transformed cells and view under a microscope

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	1 vial	-20°C	4°C
10X DMEM Solution	2 x 1.5 mL	-20°C	-20°C
Staining Solution	1 mL	-20°C	-20°C
WST Reagent III	1 vial	-20°C	4°C or -20°C
Electro Coupling Solution	1.8 mL	-20°C	4°C or -20°C

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
- Sterile dH_2O, PBS, FBS.
- Microscope
- Multi-well spectrophotometer (ELISA reader) capable of reading absorbance at 450 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 assay under sterile conditions.

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 10X DMEM Solution

1. Dilute 10X DMEM in sterile dH_2O to 1X DMEM containing 10% FBS (1X DMEM/10% FBS). For example, dilute 100 µL of 10X DMEM Solution into 900 µL dH2O 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 WST Reagent III and Electro Coupling Solution

1. Add 1.8 mL Electro Coupling Solution to the WST Reagent III vial to make the WST Working Solution.
2. WST working solution is stable for 6 months at 4°C. For long term storage (one year), aliquot and store at -20°C. Protect it from light and avoid repeated freeze/thaw cycles.

5.4 Staining 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
10X DMEM Solution	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
10X DMEM Solution	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 (optimal incubation time will vary depending upon the cell type used).

ΔNote: Assay has linear range from 10,000 to 40,000 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, 10X DMEM Solution, 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. Work quickly to prevent the agar solution from solidifying.

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 Blank (1X DMEM/10% FBS, no cells) and seven serial dilutions of cells (2-fold) in separate 1.5 mL centrifuge tubes using 1X DMEM/10% FBS as diluent.

3. Transfer 150 µL of each mixture into a separate well of a 96-well clear plate.
4. Add 35 µL of 1X DMEM/10% FBS and 15 µL of WST Working Solution into each well (Blank, Cell Standard Curve and Test Cells) and incubate at 37°C in an incubator for 4 hours.
5. Measure absorbance of all wells using a microtiter plate reader at 450 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 35 µL of 1X DMEM/10% FBS and 15 µL of WST Working Solution into each well (Blank, Cell Standard Curve and Test Cells) and incubate at 37°C in an incubator for 4 hours.
3. Measure absorbance of all wells using a microtiter plate reader at 450 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 Test Sample wells and Cell-Dose Curve wells. This is the corrected absorbance.
3. Plot the cell-dose curve (number of cells vs absorbance at 450 nm) and obtain the slope of the curve using a linear regression. The total number of transformed cells in Test Sample wells can be calculated by applying the corrected OD450 nm reading on day 6-8 to the Cell-Dose Curve (obtained on day 0), to get B (Transformed Cells Number) per well (OD450 nm = slope*cells + intercept).
4. Colony Visualization (Optional): Add 10 µl Staining Solution into each well and incubate for 60 min at 37 °C incubator with 5% CO_2. Colonies formed by transformed cells can be visualized and imaged under microscope.

8. FAQs / Troubleshooting

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

9. Typical Data

Data provided **for demonstration purposes only**.

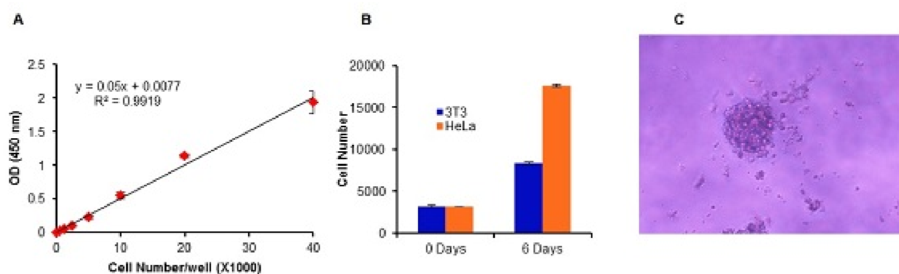


Figure 1. (A) HeLa (Human epithelial cell line from cervix adenocarcinoma) cell-dose curve; (B) Equal numbers of NIH/3T3 (Mouse embryo fibroblast cell line) and HeLa cells were seeded for the Cell Transformation Assay. After 6 days, the cell numbers were measured. Standard and Samples readings were taken 4 hours after adding WST Working Solution; (C) Image of HeLa cell colonies. HeLa cells were cultured for 6 days according to the kit protocol.

10. Notes

Technical Support

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