Cell Transformation Assay Kit (Fluorometric)

08/16

(Catalog # K922-100; 100 assays; Store at -20°C)

I. Introduction:

Transformed cells can proliferate without attaching to 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). BioVision's Cell Transformation Assay 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.

II. Applications:

- Measurement of cell transformation in response to carcinogens, oncogenes, etc.
- Assessments of chemicals that induce or inhibit cell transformation

III. Sample Type:

- Adherent or suspension cells

IV. Kit Contents:

Components	K922-100	Cap Code	Part Number
Agarose Powder	240 mg	NM	K922-100-1
DMEM Solution (10X)	2 X 1.5 ml	Clear	K922-100-2
Staining Solution	1 ml	Brown	K922-100-3
Agarose Solubilization Solution	5 ml	NM	K922-100-4
Quantitative Dye (200X)	0.1 ml	Red	K922-100-5

V. User Supplied Reagents and Equipment:

- 96-well clear tissue culture plate and 96-well white plate
- Sterile dH_2O, PBS, and FBS
- Microscope
- Multi-well spectrophotometer (ELISA reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. *Prepare reagents and perform assays under sterile conditions* (i.e. tissue culture hood/biosafety cabinet).

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

  Note: Keep the Agarose solution in a 37 °C water bath throughout cell-seeding process to prevent solidification of the agarose solution.
- **DMEM Solution (10X):** 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. Make as much as needed. Store at 4°C. Before using, warm to 37°C in a water bath.
- **Quantitative Dye (200X):** To make 1X Quantitative Dye Solution, dilute 200X Quantitative Dye with 1X PBS. For example: add 10 µl of 200X Quantitative Dye into 1.9 ml 1X PBS, mix well. *Discard the unused 1X Quantitative Dye Solution and always prepare fresh dilution.*

VII. Cell Transformation Assay Protocol:

1. Sample Preparation:

a. Preparation of Base Agarose Layer: Prepare 75 µl/well base agarose mix. For each well, mix:

1.2% agarose solution	37.5 µl
DMEM Solution (10X)	7.5 µl
100% FBS	7.5 µl
dH_2O	22.5 µl

Prepare enough Base Agarose mix for the number of experiments to be performed. Mix well. 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 min. to solidify the agarose.

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

b. Preparation of Top Agarose Layer with Cells: Prepare a stock solution of cells (1-5 $X10^6$ cells/ml) in 1X DMEM/10% FBS medium. Calculate and adjust the desired cell concentration (*see note a*) based on the number of cells per well for the assay. Prepare 75 µl/well top agarose-cell mix as follows:

1.2% agarose solution	25.0 µl
DMEM Solution (10X)	5.5 µl
FBS	5.5 µl
Cells in 1X DMEM/10% FBS	20 µl
dH_2O	19 µl

Make as much as needed. Mix by pipetting. Add 75 µl of agarose-cell mix into each well of the 96-well clear bottom tissue culture plate already containing the solidified base agarose layer. Keep the plate at 4°C for 10 min. to solidify the top agarose-cell mix. Bring the plate to room temperature by keeping it in the tissue culture hood for 10 min. Add 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*.

Notes:

a. *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.

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

c. During the process of plating the Base Agarose Layer and Top Agarose Layer with cells, 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.

d. 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.

e. 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 the microscope.

2. **Cell-Dose Curve:** On day 0: Prepare a cell-dose curve by using the stock made in step 1.b (1-5 $X10^6$ cells/ml in 1X DMEM/10% FBS medium). Arrange eight serial dilutions (2-fold) in separate 1.5 ml centrifuge tubes with 1X DMEM/10% FBS medium (150 µl). Add 50 µl of Agarose Solubilization Solution to each tube, mix and incubate cells for 15 min at RT. Transfer 20 µl of the each mixture into 96-well white plate. Add 80 µl 1X Quantitative Dye Solution to each well, protect from light and shake for 10 min on a shaker. Measure the fluorescence (RFU) of the blank and diluted cell solutions using a microtiter plate reader at Ex/Em= 480/530 nm. Subtract all readings from blank, and plot the Cell-Dose Curve.

3. **Measurement:** On day 6-8 (*at the end of the desired incubation time, step 1.b*): Carefully remove the medium above the top agarose layer by pipetting. Add 50 µl of Agarose Solubilization Solution into each well and incubate at 37 ºC incubator for 1 hr. to solubilize the agarose. 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 min. at room temperature. Measure fluorescence (Ex/Em= 480/530 nm).

4. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Cell-Dose Curve (number of cells vs $RFU_{408/530}$). Apply the sample readings (ΔRFU) to the Cell-Dose Curve (Cell number/well: $1/10^{th}$ of the original stock solutions, *step 2*) to get the number of transformed cells (B). The total number of transformed cells can be calculated using the equation $RFU_{408/530}$ = slope*cells +b.

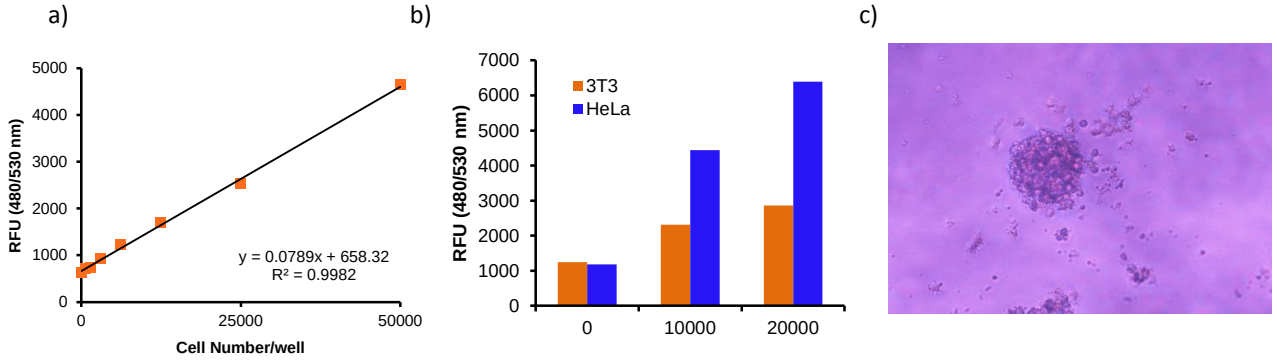

Figure: a) HeLa Cell Dose Curve; b) Anchorage-independent growth of 3T3 and HeLa cells. 3T3 and HeLa cells were serially diluted and seeded in agarose gel. Cells were solubilized and detected by the Quantitative Dye. **c**) **Image of a HeLa Colony**. HeLa cells were cultured for 7 days according to the kit protocol.

VIII. RELATED PRODUCTS:

Quick Cell Proliferation Colorimetric Assay Kit (K301)
BrdU Cell Proliferation Assay Kit (K306)
MTS Cell Proliferation Colorimetric Assay Kit (K300)
Ready-to-use Cell Proliferation Reagent, WST-1 (K304)
EZCell™ Cell Cycle Analysis Kit (K920)
Annexin V Apoptosis Kits (K101-K104)
ApoSENSOR™ ATP Cell Viability Bioluminescence Assay Kit (K254)
EZViable™ Calcein AM Cell Viability Assay Kit (Fluorometric) (K305)
Quick Cell Proliferation Colorimetric Assay Kit Plus (K302)
EZCell™ Cell Migration/Chemotaxis Assay Kits (K906-K912)
Live-Dead Cell Staining Kit (K501)
VisionBlue™ Quick Cell Viablility Fluorometric Assay Kit (K303)
Senescence Detection Kit (K320)
EZSolution™ 7-Aminoactinomycin D (2727)
StayBrite™ Highly Stable ATP Bioluminescence Assay Kit(K791)
ADP Colorimetric Assay Kit II (K356)

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