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Cell Transformation Assay Kit (Colorimetric)

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(Catalog # K921-100; 100 assays; Store at -20 °C)

I. Introduction:

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, the traditional agar method is lengthy (3-4 weeks incubation), laborious (counting colonies) and is inconsistent (due to subjective counting). **BioVision's Cell Transformation Assay** is faster, stable, and more sensitive than the traditional soft-agar assay. The kit is based on the conversion of the tetrazolium salt (WST-8) 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 wide linear range, with a maximum of 10000-400000 cells. The entire cell transformation 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 Types:

- Adherent or suspension cells

IV. Kit Contents:

Components	K921-100	Cap Code	Part Number
Agarose Powder	240 mg	NM	K921-100-1
DMEM Solution (10X)	2 x 1.5 ml	Clear	K921-100-2
Staining Solution	1 ml	Brown	K921-100-3
WST Reagent	1 vial	Green	K921-100-4
Electro Coupling Solution (ECS)	1.8 ml	Blue	K921-100-5

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagents Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge all small vials prior to opening. Read the 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 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 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 water bath.
- **WST Reagent** and **Electro Coupling Solution (ECS):** Add 1.8 ml Electro Coupling Solution to the WST Reagent vial to make the WST working solution. *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.

VII. Cell Transformation Assay Protocol:

1. Sample Preparation:

a. Preparation of Base Agarose Layer: Prepare 75 µl/well base agarose mix as following:

1.2% Agarose Solution	37.5 µl
DMEM Solution (10X)	7.5 µl
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 layer with cells, warm the plate at room temperature (RT) 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 x 10^6 cells/ml) in 1X DMEM/10% FBS medium. Calculate and adjust the desired concentration (see note a) based on the number of cells per well per assay. Prepare 75 µl/well of top agarose layer mix. Prepare as shown below:

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 for the number of test sample and cell-dose curve wells (**see Step 2 below** for cell-dose curve). Mix by pipetting. 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 min. to solidify the top agarose-cell mix. Bring the plate to RT 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 (optimal incubation time will vary depending upon the cell type used).

Notes:

- Assay has a linear range that extends from 0 to 10,000-40,000 cells, depending on the cell type used in the experiment. Adjust the maximum cell dose to avoid over-seeding.
- Include parallel well(s) with same amount of culture medium and reagents as **Blank (no cells)** for the reagent background reading.
- 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. **Work quickly to prevent the agar solution from solidifying**.
- 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.

2. **Cell-Dose Curve: On day 0,** prepare a **Cell-Dose Curve** by using the stock solution of cells made in Step 1.b (1-5 x 10^6 cells/ml in 1X DMEM/10% FBS medium). For the Cell-Dose Curve, prepare the **Blank** (1X DMEM/10% FBS, no cells) and seven 2-fold serial dilutions of stock solution of cells in separate 1.5 ml microcentrifuge tubes using 1X DMEM/10% FBS as diluent. Transfer 150 µl of the each dilution into a 96-well clear plate. Add **35 µl of 1X DMEM/10% FBS** and **15 µl of WST working solution** into each well (**Blank** and **Cell-Dose Dilutions**) and incubate at 37 °C incubator for 4 hrs. Measure the absorbance of all wells at 450 nm using a microtiter plate reader.
3. **Measurement: On day 6-8** (that is at the end of the desired incubation time, see **Step 1.b**), carefully remove the medium on top of the top agarose layer by pipetting. Add 35 µl of 1X DMEM/10% FBS and 15 µl of WST working solution into each well (**Blank** and **Test Sample wells**) and incubate at 37 °C incubator for 4 hrs. Measure the absorbance of all wells using a microtiter plate reader at 450 nm ($OD_{450\ nm}$).
4. **Calculation:** Subtract the **Blank (no cells)** reading from all **Test Sample wells** and **Cell-Dose Curve wells**. 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 $OD_{450\ nm}$ reading on day 6-8 to the Cell-Dose Curve (obtained on day 0), to get B (Transformed Cells Number) per well ($OD_{450\ nm}$ = slope*cells + intercept).

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.

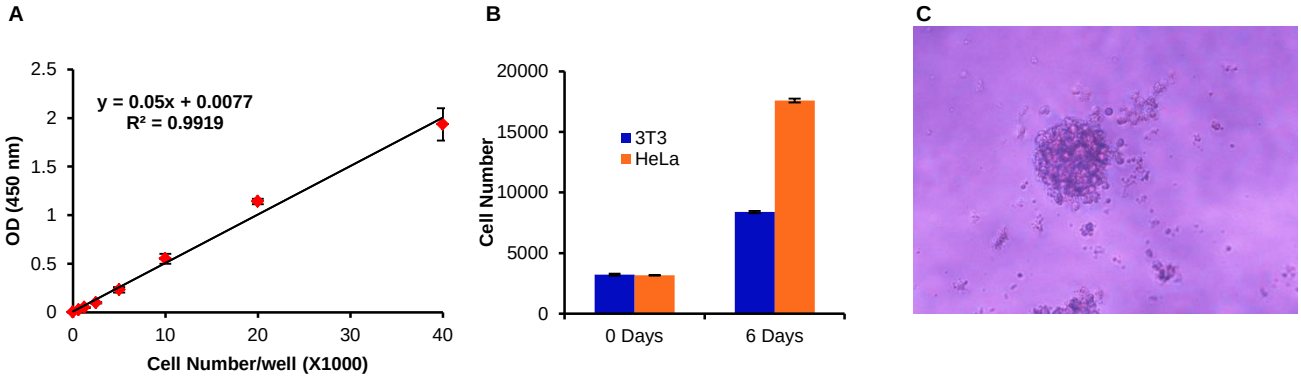

Figure: A. HeLa Cell-Dose Curve (obtained at day 0). **B.** Equal number of 3T3 and HeLa cells are seeded for cell transformation assay. After 6 days, the cell numbers were measured. Standard and Samples readings were taken 4 hrs after adding WST working solution **C.** Image of HeLa cell colonies. HeLa cells were cultured for 6 days according to the kit protocol.

VIII. Related Products:

Cell Transformation Assay Kit (K922)
ApoBrdU Red DNA Fragmentation Assay Kit (K404)
ApoDIRECT DNA Fragmentation Assay Kit (K402)
Enhanced Apoptotic DNA Ladder Detection Kit (K130)
Genomic DNA Isolation Kit (K281)
Link-FAST™ 5 Minutes DNA Ligation Kit (K902)
ApoBrdU DNA Fragmentation Assay Kit (K401)
ApoBrdU-IHC DNA Fragmentation Assay Kit (K403)
Apoptotic DNA Ladder Isolation Kit (K170)
Quick Apoptotic DNA Ladder Detection Kit (K120)
Mitochondrial DNA Isolation Kit (K280)
DNA Damage Quantification Colorimetric Kit (K253)

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