# Cell Transformation Assay Kit (Colorimetric) (#BN01095)

**(Catalog # BN01095; 100 assays; Store at -20°C)**

## I. Introduction:

The 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). Assay Genie'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 from 10000-400000 cells. The entire Cell Transformation Assay can be finished within 7-8 days.

## II. Application:

- 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 | BN01095 | Cap Code | Part Number |
|---|---|---|---|
| Agarose Powder | 240 mg | NM | BN01095-1 |
| DMEM Solution (10X) | 2 X 1.5 ml | Clear | BN01095-2 |
| Staining Solution | 1 ml | Brown | BN01095-3 |
| WST Reagent | 1 vial | Green | BN01095-4 |
| Electro Coupling Solution (ECS) | 1.8 ml | Blue | BN01095-5 |

## 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 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 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 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 1 X 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 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 for the number of sample and cell-dose curve wells. 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 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 10000 to 40,000 cells, depending on the cell type used in the experiment. Adjust the cell numbers to avoid over-seeding.

**b.** Perform 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 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.

**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 microscope.

2. **Cell-Dose Curve:** On day 0: Prepare a cell-dose curve by using the stock made in step 1.b (1-5 X $10^6$ cells/ml in 1 X DMEM/10% FBS medium). Arrange 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. Transfer 150 µl of the each mixture into 96-well clear plate. 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 incubator for 4 hrs. Measure absorbance using a microtiter plate reader at 450 nm.
3. **Measurement:** On day 6-8 (*at the end of the desired incubation time, 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, Cell Standard Curve and Test Cells) and incubate at 37°C incubator for 4 hrs. Measure absorbance using a microtiter plate reader at 450 nm.
4. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Cell-Dose Curve. Apply the corrected sample reading OD450 to Standard Curve to get B Transformed Cells Number/well. The total number of transformed cells can be calculated.

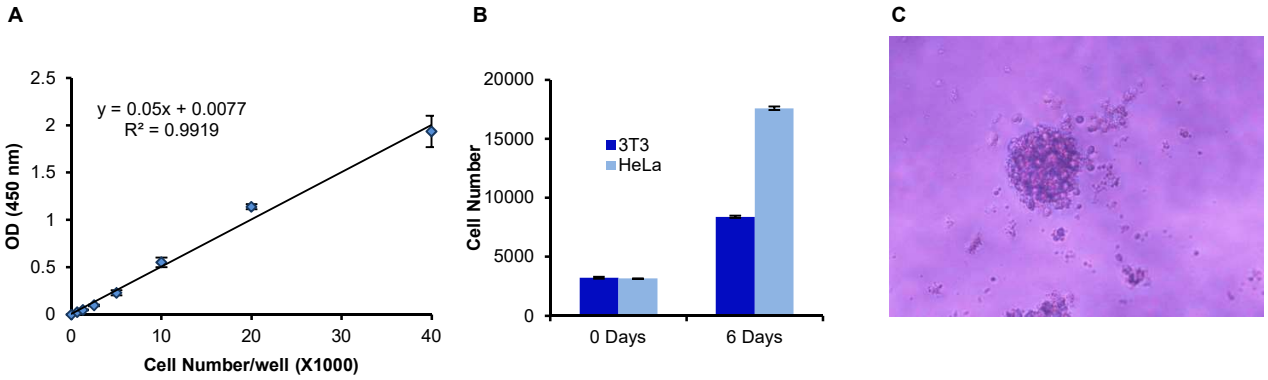


**Figure: A.** HeLa Cell Dose Curve. **B.** Equal number of 3T3 and HeLa cells are seeded for Cell Transformation Assay. After 6 days, the cell numbers were measured. Standard and Samples readings were taken 4 hours after adding WST working solution **C.** Image of HeLa cell colonies. HeLa cells were cultured for 6 days according to the kit protocol.

***FOR RESEARCH USE ONLY! Not to be used on humans.***