3D Cell Culture HTS Cell Viability Complete Assay Kit (BN01109)

(Catalog BN01109; 100 assays; Store at -20°C)

I. Introduction:

Three-dimensional (3D) cell cultures are artificially-created environments in which cells are permitted to grow or interact with their surroundings in a 3D fashion. 3D cell cultures improve the function, differentiation and viability of cells and recapitulate *in vivo* microenvironment compared to conventional 2D cell cultures. 3D matrices provide a physiologically relevant screening platform, by mimicking the *in vivo* responses, for many cell types including cancer and stem cells in developmental morphogenesis, pharmacology, drug metabolism and drug toxicity studies. Quantification of number of viable cells is an indispensable tool in *in vitro* screening in these studies. Calcein AM is a non-fluorescent, hydrophobic compound that easily penetrates intact and live cells, and has been widely used to assess cell viability and proliferation in Cell Biology research. However, with the use of 3D matrices, some proteases-based dissociation methods don't completely dissolve the matrices and cell aggregates, which could alter the result in quantitative *in vitro* assays such as viability assessment. Assay Genie's 3D Cell Culture Cell Viability Complete Assay Kit provides a standardized fluorometric method for sensitive quantification of viable cells that can detect as low as 50 viable cells in each well and can be measured at Ex/Em = 485/530 nm. The measured fluorescence intensity is proportional to the number of viable cells. Further, as a complete set, the kit comes with an optimized and gentle non-enzymatic dissociation solution for the recovery of viable and dead cells from spheroids in matrices and scaffolds. This assay kit provides an easy-to-use, non-radioactive, and high-throughput method for characterizing and screening cell viability, cytotoxicity and apoptosis.

II. Application:

- Matrix and spheroid dissociations from 3D cell culture for cell growth assessment
- Measurement of cell viability in response to growth factors, cytokines, mitogens and nutrients
- Analysis of cytotoxic/cytostatic compounds that affect cell growth and spheroid formation, such as anticancer drugs, toxic agents and other pharmaceuticals

III. Sample Type:

- Proliferating and non-proliferating cells in 3D culture Matrices and Scaffolds

IV. Kit Contents:

Components	BN01109	Cap Code
Matrix Dissociation Saline Solution	40 ml	NM
Viability Assay Buffer	100 ml	NM
Calcein AM	1 vial	Green

V. User Supplied Reagents & Equipment:

- 3D Cell culture matrix and scaffold
- 96-well White plate with clear bottom (sterile, cell culture grade)
- Hemocytometer or automated cell counter for Standard Curve (optional)
- Cell Culture Media
- DMSO

VI. Reagents Preparation and Storage Conditions:

Store kit at -20°C, protected from light. Assay is performed under sterile conditions. Read entire protocol before performing the experiment.

- **Matrix Dissociation Saline Solution** and **Viability Assay Buffer:** Store at -20°C. Thaw and keep at 4°C before use. Stable for six months after the first thaw.
- **Calcein AM:** Resuspend in 100 µl anhydrous DMSO (not provided) as needed. Aliquot and store -20°C. Use within 2 months.

VII. Cell Viability Assay Protocol:

1. **3D Cell Culture:** Grow cells in appropriate media and culture conditions. Adherent cells should be cultured to ~80% confluency. For both adherent and suspension cells, harvest cells and centrifuge at 1,000 x g, for 5 min. Re-suspend the cell pellet in Assay Buffer and count the number of cells using a hemocytometer or an automated cell counter. Re-suspend cells in 1 ml of media yielding a concentration of 2 x 10^6 cells/ml. For 3D Cell Culture: in a 96 well-plate, add 500 µl of re-suspended cells to 5 ml of matrix of interest. Mix gently by pipetting and add 50 µl of cell matrix mixture to each well to get 10,000 cells per well.

   Notes:

   a. Matrix is not provided; follow the appropriate protocol for matrix preparation. For 3D Cell culture environments, we recommend Assay Genie's 3D Cell culture matrices (Alginate, Basement Membrane, or Duo Matrix Kits).

   b. It is recommended that cells are allowed to form spheroids for at least 3 days before performing any drug screening study.

2. **Matrix Dissociation**: After cells formed spheroids in appropriate matrix, and/or drug screening study is completed, remove all media by pipetting and add 200 µl of Matrix Dissociation Saline Solution. Incubate at RT for 5-10 min. and then pipet up and down with 1 ml tip until matrix is dissolved.

Notes:

a. If matrix doesn't completely dissolve in well, add additional 100 µl of Matrix Dissociation Solution and incubate for another 10 min.

b. Matrix Dissociation Solution works best on natural animal-based and plant-based matrices and scaffolds. *Synthetic polymers have not been tested with this kit.*

3. **Neutralization:** Add 100 µl of Viability Assay Buffer and centrifuge the plate at 1,000 x g, for 5 min at 4°C. Remove all liquid solution from each well without disrupting the cell pellet. Resuspend cells in 50 µl of fresh Viability Assay Buffer.
4. **Background Controls (BC) Preparation***:* For Cell-only control well, in one well of untreated cells, resuspend cells in 100 µl of Viability Assay Buffer.
5. **Cell Viability Standard Curve***:* Take a fresh new split of cells (of the same clone/type, but not from 3D culture or drug screening assay). Harvest cells and centrifuge at 1,000 x g, for 5 min. Resuspend the cell pellet in Assay Buffer and count the number of cells using a hemocytometer or an automated cell counter. Re-suspend cells in 1 ml of Viability Assay Buffer at the concentration of 2 x 10^6 cells/ml. add 100 µl of re-suspended cells to 500 µl of buffer. Mix gently by pipetting, and add **0, 10, 20, 30, 40, 50 µl** of cell mixture to six consecutive wells to get **0, 4k, 8k, 12k, 16k, 20k** cells per well. Bring volume up to 50 µl with blank Viability Assay Buffer.

   Note: Each cell type has a different measurement for Standard curve Calculation. It is recommended to graph a new standard curve each time a new cell type is used.
6. **Staining & Detection:** For 100 wells, dilute 24 µl of Calcein AM dye in 6 ml of Viability Assay Buffer (or 1:250 dilution). Add 50 µl of Calcein AM working solution to wells from **steps VII-3** (and Standard Curve, **step VII-5**, *optional*), making the total volume of 100 µl for each well. For **BC** well, *do not* add any Calcein AM dye or solution. Incubate the plate at 37°C for 30 min & measure fluorescence **(Ex/Em = 485/530 nm).**

   Notes:

   a. Use diluted Calcein AM immediately. Don't store the diluted Calcein AM for more than 4 hr.

   b. Appropriate incubation time depends on the individual cell type and cell concentrations used. Therefore, it is recommended to determine the optimal condition for each experiment.
7. **Calculation:** For Standard Curve, subtract 0 Standard reading from all readings and plot the Standard Curve. For assay wells, subtract the background control reading (from **step VII-4)** from all sample readings. Apply ∆RFU to the Standard curve to get the number of viable cells in each well.

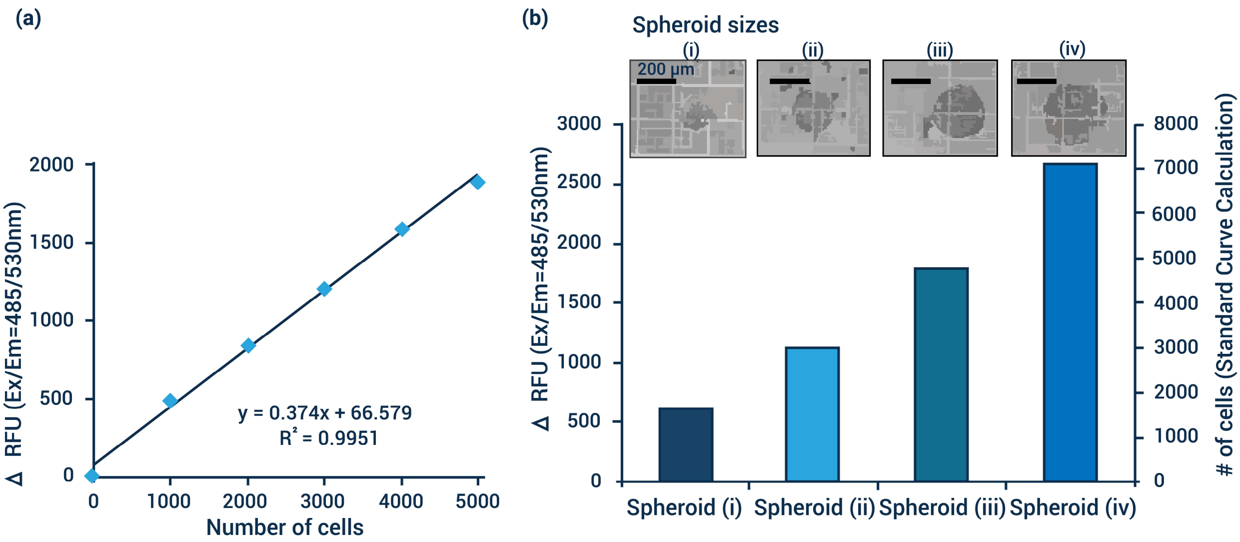

Figure 1: (a) Standard curve for number of viable HepG2 cells. Standard 0 reading was subtracted from all readings. **(b)** HepG2 Cells were cultured in 3D cell Culture BME Matrix for 21 days and spheroids were formed. Before dissociation, each spheroid was isolated from matrix and images were captured for sizing purposes (spheroid sizes i-iv, scale bar = 200 µm). Next, spheroids were dissociated, stained with Calcein AM, and measured following Viability Assay protocol (∆ RFU data are shown in bar graph).

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