ab284562 – 3D Cell Culture HTS Cell Viability Complete Assay Kit

For the:

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

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284562

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Matrix Dissociation Saline Solution	40 mL	-20°C
Viability Assay Buffer	100 mL	-20°C
Calcein AM	1 vial	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 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

Reagent Preparation

- Assay is performed under sterile conditions

Matrix Dissociation Saline Solution and Viability Assay Buffer: Thaw and keep at 4°C before use. Stable for 6 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.

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 106 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.

   Δ Notes:
   a. *Matrix is not provided; follow the appropriate protocol for matrix preparation.*
   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.

   Δ Notes:
   a. *If matrix doesn't completely dissolved 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.

Background Controls (BC) Preparation:
For Cell-only control well, in one well of untreated cells, resuspend cells in 100 µL of Viability Assay Buffer.

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 106 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.

Δ 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.*

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 (and Standard Curve, 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).

Δ 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.*

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 all sample readings. Apply ΔRFU to the Standard curve to get the number of viable cells in each well.

Technical Support

For all technical or commercial enquiries please go to:

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