ab284534 – 3D Culture HTS Cell Viability Assay Kit (Colorimetric)

For the sensitive quantification of viable cells.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284534

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, store the kit components as per the respective temperatures mentioned below. Use kit within 6 months.

Materials Supplied

Item	Quantity	Storage Condition
Matrix Dissociation Saline Solution	40 mL	-20°C
Viability Assay Buffer	50 mL	-20°C
WST Concentrate	60 µL	-20°C

Materials Required, Not Supplied

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

- Cells
- 3D cell culture matrix and scaffold
- 96 well clear plate (Sterile, cell culture grade)
- Hemocytometer or automated cell counter
- Cell culture media
- Absorbance plate reader
- Centrifuge that can accommodate a 96-well plate

Reagent Preparation

- Assay should be performed under sterile conditions.

Matrix Dissociation Saline Solution and Viability Assay Buffer: Thaw at room temperature (RT) and store at 4°C when not in use (protect from light). Stable for six months after the first thaw when stored at 4°C.

WST Concentrate (40X): Provided as a 40X concentrated solution. Divide into aliquots as desired and store at -20°C, protected from light. Avoid repeated freeze/thaw cycles.

Cell Viability Assay Protocol

3D Culture:

1. Matrix for 3D culture is not provided. Follow the appropriate protocol for matrix preparation of your choice.
2. It is recommended that cells are allowed to form spheroids for at least seven or more days before performing any drug screening study.

Matrix Dissociation:

1. After cells have formed spheroids in the appropriate matrix, and/or drug screening study is completed, remove all the media using a pipette tip and add 200 µl of Matrix Dissociation Saline Solution.
2. Incubate at RT for 5-10 min and pipette up and down with 1 ml tip until the matrix is dissolved.
   Δ Note: *If matrix doesn't completely dissolve, add an additional 50 µl of Matrix Dissociation Saline Solution and incubate for another 10 minutes.*
   Δ Note: *Matrix Dissociation Saline Solution works best on natural animal-based, and plant based matrices and scaffolds. Synthetic polymers have not been tested with this kit.*

Neutralization:

1. Add 50 µl of Viability Assay Buffer and centrifuge the plate at 1,000 x g, for 5 minutes at 4°C.
2. Carefully remove all of the liquid from each well without disrupting the cell pellet.
3. Resuspend cells in 150 µl of fresh Viability Assay Buffer.

Cell Viability Standard Curve:

1. Obtain a flask actively growing, 70-85% confluent, cells (of the same clone and type, but not from 3D culture or drug screening assay).
2. Harvest the cells and centrifuge at 300 x g, for 5 minutes at 4°C.
3. Resuspend the cell pellet in a small volume (<1 ml) of Viability Assay Buffer and count the number of cells using a hemacytometer or an automated cell counter.
4. Resuspend the cells in Viability Assay Buffer to a concentration of 2.5 x 10^6 cells/ml.
5. Mix gently by pipetting, then add (in duplicate wells) 0, 5, 10, 20, 30, 50, 100 µl of the cell mixture to seven consecutive wells to get 0, 12.5K, 25K, 50K, 75K, 125K, 250K cells per well.
6. Bring the volume up to 150 µl with Viability Assay Buffer.

Δ Note: *Each cell type must have a unique Standard curve. It is recommended to prepare a new Standard Curve for each cell type used.*

Staining and Detection:

1. Dilute the WST Concentrate dye at a 1:10 ratio in Viability Assay Buffer to prepare the WST dye working solution (for example, for 100 wells, mix 0.5 ml of WST Concentrate dye and 4.5 ml of Viability assay Buffer).
2. Add 50 µl of WST dye working solution to wells from the Neutralization step and Cell Viability Curve step, making the total volume of 200 µl for each well.
3. Incubate the plate at 37°C.
4. Read the plate at 30, 60, 90, 120, 150, and 180 min by measuring absorbance at 460 nm.

Δ Note: *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 by reading every 30 minutes until the Standard Curve and 3D cultured cells are in the desired absorbance range.*

Calculation:

1. For Standard Curve, subtract 0 Standard reading from all readings and plot the Standard Curve.
2. For assay wells, subtract the 0 standard reading from all Sample readings.
3. Apply the absorbance readings to the Standard Curve to determine the number of viable cells in each well.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

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