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3D Culture HTS Cell Viability Assay Kit (Colorimetric)

(Catalog # K2032-100; 100 assays; Store at -20°C) *12/19*

I. Introduction:

Three-dimensional (3D) cell cultures are artificially-created environments, where cells are allowed to grow or interact with their surroundings in a 3D fashion. 3D cell cultures improve the function, differentiation and viability of cells and recapitulate the *in vivo* microenvironment as 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. This is specifically important in developmental morphogenesis, pharmacology, drug metabolism and drug toxicity studies. Additionally, quantification of the number of viable cells is an indispensable tool in *in vitro* screening in these studies. However, with the use of 3D matrices, some protease-based dissociation methods don't completely dissolve the matrices or the cell aggregates, which may alter the result of the *in vitro* viability assessment quantitatively. **BioVision's 3D Culture Cell Viability Assay Kit** provides a standardized colorimetric method for sensitive quantification of viable cells that can detect as high as 250,000 viable cells and as low as 1000 viable cells in each well. The absorbance is measured at 460 nm. The measured intensity is proportional to the number of viable cells. Further, 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, high-throughput method for characterizing and screening cell viability and cytotoxicity**.**

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	K2032-100	Cap Code	Part Number
Matrix Dissociation Saline Solution	40 ml	NM	K2032-100-1
Viability Assay Buffer	50 ml	NM	K2032-100-2
WST Concentrate	600 µl	Green	K2032-100-3

V. User Supplied Reagents and Equipment:

- 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

VI. Reagent Preparation and Storage Conditions:

Store kit at -20°C, protected from light. Assay should be performed under sterile conditions. Read the entire protocol before performing the assay procedure.

- **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.

VII. Cell Viability Assay Protocol:

1. 3D Cell Culture:

a. Matrix for 3D culture is not provided. Follow the appropriate protocol for matrix preparation. For 3D Cell culture environments, we recommend BioVision Cat. # K518, K519 or K520, 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 7+ days before performing any drug screening study.

2. Matrix Dissociation: 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. Incubate at RT for 5-10 min and pipette up and down with 1 ml tip until the matrix is dissolved.

Notes:

- If matrix doesn't completely dissolve, add an additional 50 µl of Matrix Dissociation Saline Solution and incubate for another 10 min.
- 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.

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

4. Cell Viability Standard Curve*:* Obtain a flask actively growing, 70-85% confluent, cells (of the same clone and type, but not from 3D culture or drug screening assay). Harvest the cells and centrifuge at 300 x *g*, for 5 min at 4°C. Resuspend the cell pellet in a small

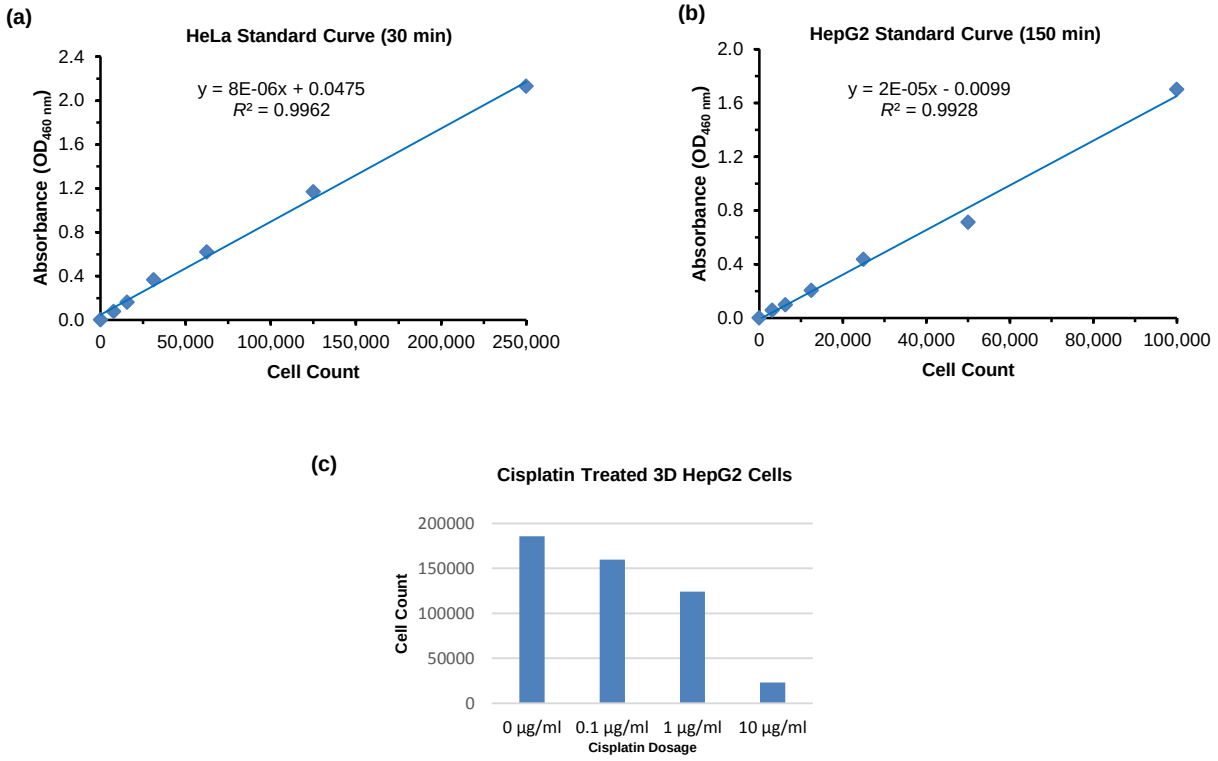
volume (<1 ml) of Viability Assay Buffer and count the number of cells using a hemocytometer or an automated cell counter. Re-suspend the cells in Viability Assay Buffer to a concentration of 2.5 x 10^6 cells/ml. Mix gently by pipetting, then add (in duplicate wells) **0, 5, 10, 20, 30, 50, 100 µl** of the cell mixture to seven consecutive wells to get **0, 12.5K, 25K, 50K, 75K, 125K, 250K** cells per well. Bring the volume up to 150 µl with Viability Assay Buffer.

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

5. **Staining & Detection:** 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). Add 50 µl of WST dye working solution to wells from **steps VII-3** (and Standard Curve, **step VII-4**), making the total volume of 200 µl for each well. Incubate the plate at 37°C. Read the plate at 30, 60, 90, 120, 150, and 180 min by measuring absorbance at 460 nm.

   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 min until the Standard Curve and 3D cultured cells are in the desired absorbance range.

6. **Calculation:** For Standard Curve, subtract 0 Standard reading from all readings and plot the Standard Curve. For assay wells, subtract the 0 standard reading (from **step VII-4)** from all Sample readings. Apply the absorbance readings to the Standard Curve to determine the number of viable cells in each well.



Figures: (a) Standard Curve for viable HeLa cells (0 to 250,000 cells). **(b)** Standard Curve for viable HepG2 cells (0 to 100,000 cells). **(c).** Viable cells (HepG2) from 3D culture following 48 hour treatment with Cisplatin. Cells were cultured in 3D Cell Culture BME Matrix (Biovision Cat. #K518) for 18 days to form spheroids. Spheroids were treated as described above and the viable cells determined according to the kit protocol.

VIII. Related Products:

3D Cell Culture Matrix Alginate (K517)	3D Cell Culture Matrix Duo-Matrix (K519)
3D Cell Culture Matrix BME (K518)	Quick Cell Proliferation Colorimetric Assay Kit (K301)
3D Cell Culture Scaffold (K990)	BrdU Cell Proliferation Assay Kit (K306)
Trypan Blue (0.4%) (1209)	3D Cell Culture HTS Cell Viability Complete Assay Kit (K948)

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