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3D Cell Culture Non-Enzymatic Cell Harvesting Kit

08/16

(Catalog #K982-100; 100 samples; 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. However, when it comes to cell passaging, or studying biochemical processes and protein analysis, there are challenges with separating intact cells from extra-cellular proteins comprising the 3D matrices. While proteases (such as trypsin and accutase) are commonly used to degrade these matrices, it is not recommended in some cases where cells are sensitive to protease digestion; these proteases may modify cellular proteins and signaling or cell surface, thus altering physiological assessments. Further, some proteases-based dissociation methods do not completely dissolve the matrices. BioVision's 3D Culture Non-Enzymatic Cell Harvesting Kit provides an optimized and standardized saline-based solution for the isolation of cells and spheroids from matrices (especially for BioVision's 3D Cell Culture Kits: K517, 518, 519) with high viability rate for subsequent biochemical, protein and cell-based analysis.

II. Application:

- Matrix and cell /spheroid dissociations
- Cell-harvesting for usage in cell-based assay, biochemical and protein analysis

III. Sample Type:

- Matrices
- Adherent and suspension spheroids and cells

IV. Kit Contents:

Components	K982-100	Cap Code	Part Number
Matrix Dissociation Saline Solution	40 ml	NM	K982-100-1
Neutralization Buffer	100 ml	NM	K982-100-2

V. User Supplied Reagents & Equipment:

- 1.5 ml Eppendorf tubes (clear and sterile)
- 96-well clear microplate

VI. Reagents Preparation and Storage Conditions:

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

- **Matrix Dissociation Saline Solution** and **Neutralization Buffer:** Store at -20°C. Thaw and keep at 4°C before use. Stable for six months after the first thaw.

VII. Non-Enzymatic Cell Harvesting Protocol:

1. **Matrix Dissociation:** *The following protocol has been optimized for 100 samples that can be processed in a 96- microplate. For bigger wells, the numbers of processed samples will change. Refer to table in step VII-3 for suggested volumes if using a different plate).* In a clear 96-well microplate, grow cells in appropriate 3D matrices and conditions. Remove all media from wells in the 96-well plate, and add 250-300 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. Transfer the cells and solution to 1.5 ml Eppendorf tubes.

   Notes:

   a. If matrix doesn't completely dissolve in well, transfer samples to clear Eppendorf tubes, add additional 100 µl of Matrix Dissociation Solution and incubate for another 5-10 min. Vortex at low setting if needed.

   b. Matrix Dissociation Buffer works best on natural animal and/or plant-based matrices. Synthetic polymers have not been tested with this kit.

2. **Neutralization:** To neutralize the Matrix Dissociation Saline Solution, add 700 µl of Neutralization Buffer to each tube, invert tube a few times to mix, and centrifuge at 1,000 x g, for 5 min at 4°C. Remove the solution carefully without disrupting the cell pellets. Resuspend cell pellet in Neutralization Buffer or buffer and media of your preference, for use in assay of interest.

   Notes:

   a. For biochemistry or protein analysis, use ice-cold Matrix Dissociation Saline Solution and Neutralization Buffer, and keep samples on ice.

   b. For lysate preparation and storage: add ice-cold Cell lysis buffer (BioVision's Cell Lysis Buffer 1067-100, -400 or alternative) to cell pellets, snap freeze samples and store at -80°C.

   c. For live-cell storage (freeze down cells): resuspend cell pellets in appropriate cryopreservation solution and store at -80°C.

3. Plate optimization *(optional, plates are not provided)*: optimized volumes are indicated in table below

Plate type	96-well	48-well	24-well	12-well	6-well
Matrix Dissociation Solution (ml/sample)	0.3	0.6	1.5	3	5
Neutralization buffer (ml/sample)	0.7	1.4	3	6	12

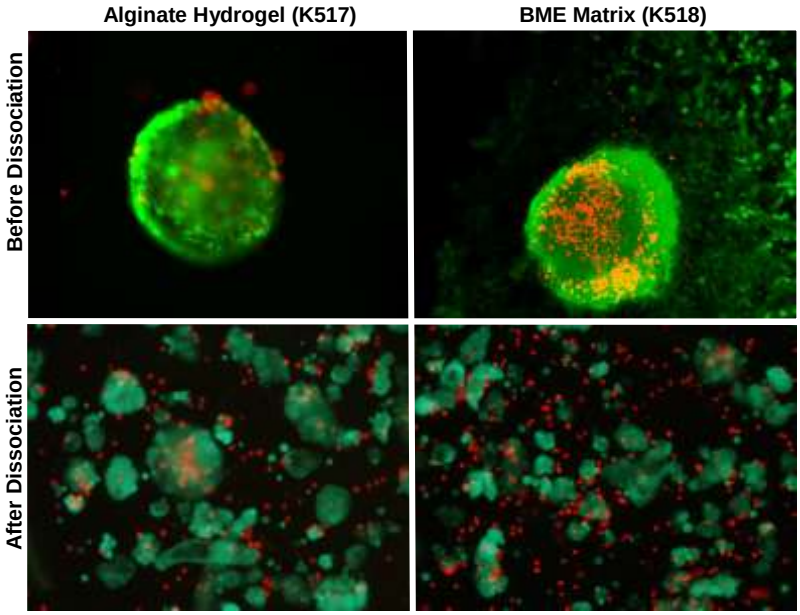

Figure: Dissociation of HepG2 cells from 3D cell culture Alginate Hydrogel (K517) or BME Matrix (K518): Cells were cultured and formed spheroids in Alginate Hydrogel or BME Matrices for 21 days. Cells were then dissociated from matrices and harvested according to protocol above. Analysis using Calcein AM staining (green, for live cells), and Ethidium Homodimer-1 dye (red, for apoptotic cells) suggested that cell viability is not affected after performing harvesting protocol. ***Note:*** *Calcein AM (Cat. No. 1755) and Ethidium Homodimer-1 are not included in the kit.*

VIII. RELATED PRODUCTS:

3D Cell Culture Matrix Alginate (K517)
3D Cell Culture Matrix BME (K518)
Cytosol/Particulate Separation Kit (K267)
Calcein AM (1755)
3D Cell Culture Matrix Duo-Matrix (K519)
Quick Cell Proliferation Colorimetric Assay Kit (K301)
BrdU Cell Proliferation Assay Kit (K306)
Ethidium bromide (1203)

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