Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

3D Cell Culture Matrix BME Kit

06/16

(Catalog #K518-100; 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. BioVision offers 3D cell culture matrices, including Basement Membrane Matrix (BME, animal-based), Alginate Hydrogel (plant-based) and proprietary Biovision's Duo-Matrix Mix, to meet the needs and requirements of various research fields. BioVision's 3D Culture Matrix Kits provide a standardized, yet user friendly and adaptable to high-throughput strategy for setting up spheroid formations, 3D cell cultures and pharmacological studies.

II. Application:

- Spheroid formation assays
- Adaptable to any 3D cell culture based drug screening studies

III. Sample Type:

- Adherent and suspension cells

IV. Kit Contents:

Components	K518-100	Cap Code	Part Number
BME Matrix	5.0 ml	NM	K518-100-1
Wash Buffer	100 ml	NM	K518-100-2

V. User Supplied Reagents & Equipment:

- Cell Culture Media
- 96 well plate (sterile, clear-bottom)
- Microscope
- Matrix Dissociation Buffer (Cat No. M1090)

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.

- **BME Matrix:** Aliquot and store at –20°C. Avoid multiple freeze/thaw. Thaw and keep on ice before use. Use within two months.
- **Wash Buffer:** Store at -20°C or 4°C. Stable for six months after the first thaw. Bring to room temperature (RT) before use.

VII. 3D Cell Culture Protocol:

1. **Cells:** Grow cells in appropriate media and culture conditions. Adherent cells should be cultured to ~80% confluency. Harvest cells and centrifuge at 1,000 x g, for 5 min. Resuspend the cell pellet in Wash Buffer and count the number of cells using a hemocytometer or an automated cell counter. Re-suspend cells in 500 µl of media at the concentration of 2 x 10^6 cells/ml, and keep cells on ice.
2. **Matrix Preparation:** Thaw Matrix and perform the assay on ice at all time. For a 96 well-plate, add 500 µl ml of resuspended cells from step VII-1 to 4.5 ml of thawed BME Matrix. Mix gently by pipetting, and add 50 µl of cell mixture to each well to get 10,000 cells per well. To solidify the matrix, incubate the entire plate in 37°C with cell and matrix mixture for 30 min. After the incubation time, add 200-250 µl of appropriate media and allow cells to grow and form spheroids in 37°C incubator for a set amount of days depending on experimental set up. Change media every 2-3 days.

   Note:

   a. For a scaled-down experiment to 10 wells, add 55 µl of cells in media (2 x 10^6 cells/ml) to 495 µl of BME Matrix. Next, add 50 µl of cell mixture to each well to get 10,000 cells per well.

   b. Cells typically form spheroids in matrix after 1 week.

   c. BME Matrix remains stable for up to 3 weeks in culture. Vacuum removal of buffer or media could aspirate some or the entire matrix and can cause loss of samples. To avoid rupture of matrix, removal of buffer and media, by carefully pipetting from side of well, is strongly recommended.
3. **Matrix Dissociation (optional):** Matrix Dissociation Buffer **(**Cat No. M1090**)** is not provided. Add 200-250 µl of Matrix Dissociation Solution. Incubate at RT for 5-10 min. and then pipet up and down with 1 ml tip until matrix is dissolved. Move the cells and solution to 1.5 ml Eppendorf tubes. To neutralize the Matrix Dissociation Solution, add 1 ml of Wash Buffer to each tube and centrifuge at 1,000 x g, for 5 min. Resuspend cells in media for use in assay of interest.

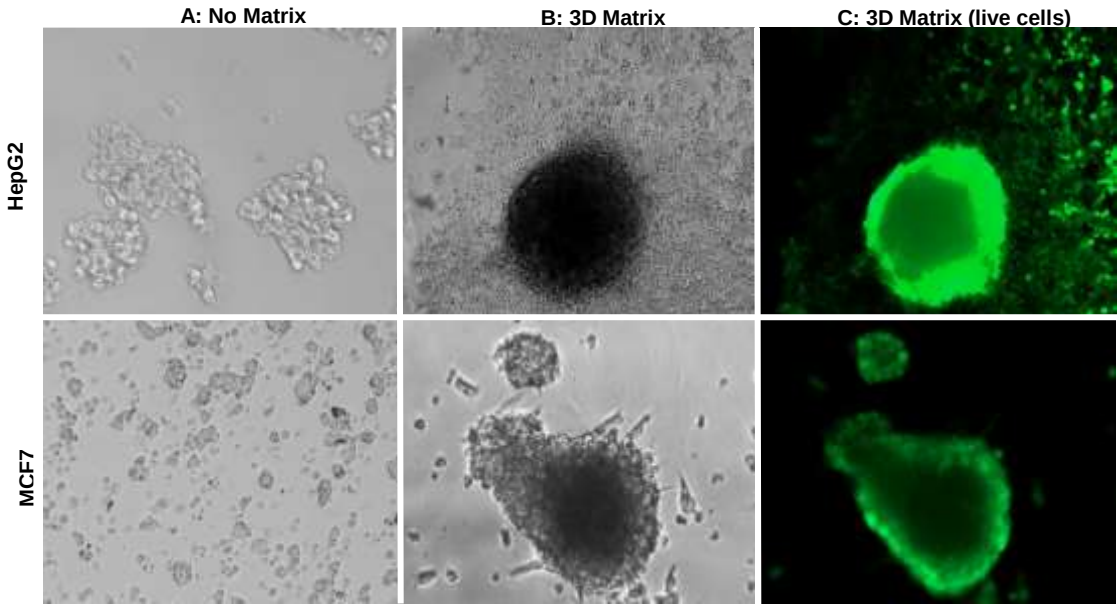

Figure: HEPG2 and MCF7 cells in No Matrix (A) and 3D BME Matrix (B). Cells were cultured in BME Matrix for 21 days, and successfully formed spheroids. Media was changed every 2-3 days as per protocol. The Calcein AM staining (C) indicates that cell viability is not affected while culturing in matrix for a long period of time. ***Note:*** *Calcein AM is not included.*

VIII. RELATED PRODUCTS:

3D Cell Culture Matrix Alginate Hydrogel (K517)
Mitochondria/Cytosol fraction Kit (K256)
Cytosol/Particulate Separation Kit (K267)
Matrix Dissociation Buffer (M1090)
3D Cell Culture Matrix Duo-Matrix (K519)
Quick Cell Proliferation Colorimetric Assay Kit (K301)
BrdU Cell Proliferation Assay Kit (K306)
Calcein AM (1755-1000)

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