*FOR RESEARCH USE ONLY!*

# 3D Cell Culture Matrix Alginate Hydrogel Kit

*01/17*

**(Catalog #K517-100; 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. Our 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 | K517-100 | Cap Code | Part Number |
|---|---|---|---|
| Alginate Hydrogel Matrix | 5 ml | NM | K517-100-1 |
| Cross-linking Solution | 25 ml | NM | K517-100-2 |
| Wash Buffer | 100 ml | NM | K517-100-3 |

## 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.

- **Alginate Hydrogel Matrix:** Aliquot and store at -20°C. Avoid multiple freeze/thaw. Use within two months. Thaw and keep on ice before use.
- **Cross-linking Solution:** Store at -20°C. Thaw and keep at 4°C before use. Stable for six months after the first thaw.
- **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. For 96 well-plate, add 500 µl of resuspended cells to 4.5 ml of thawed Alginate Hydrogel Matrix at RT. Mix gently by pipetting, and add 50 µl of cell mixture to each well to get 10,000 cells per well.

   **Note:** 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 Alginate Hydrogel Matrix. Next, add 50 µl of cell mixture to each well to get 10,000 cells per well.

2. **Matrix Preparation**: To solidify the matrix, add 250 µl of ice-cold Cross-linking Solution to each well. Incubate at RT for 5-10 min until matrix is formed (clear gel in liquid solution). After the matrix solidification, remove all liquid by pipetting, and Alginate Hydrogel remains in the well. Wash three times with 200 µl of Wash Buffer. 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.** Cells typically form spheroids in matrix after 1 week. 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.

   **b.** Removal of media (i.e. by careful pipetting) 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.

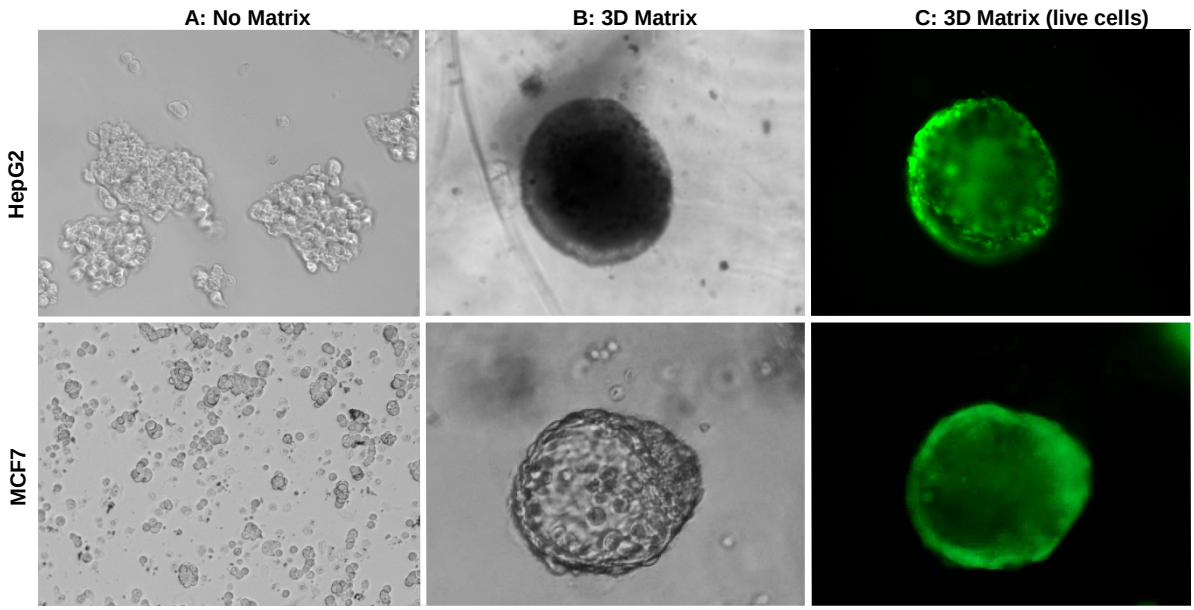


**Figure: HepG2 and MCF7 cells in No Matrix (A) and 3D Alginate Hydrogel Matrix (B).** Cells were cultured in Alginate Hydrogel Matrix for 21 days, and successfully formed spheroids. Media was changed every 2-3 days as per protocol. The Calcein AM staining (C) indicates that cell viability is not affected while culturing in matrix for a long period of time. ***Note:*** *Calcein AM (Cat. No. 1755) is not included in the kit.*

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