Biozellen®3D Cell Organoid Culture Hydrogel Kit

Catalog No. B-P-00003-2、B-P-00003-4、B-P-00003-10
Specification 2ml-kit、4ml-kit 、10ml-kit
Storage Store at-20℃. Keep for one years.

Product Description

Biozellen®3D Cell Organoid Culture Hydrogel kit is a complete set of reagents designed for the microenvironments for 3D organoid culture and related applications. The complete control of biomolecular modifications and gel stiffness of this easy-to-use kit allow a great variety of cell culture applications. The polymers can generate hydrogels at fast gelation rate. It is recommended to read the User Guide carefully before setting up the 3D culture gels.

(**The hydrogel material is of plant origin, no animal origin**)

Applications

- Organoid formation assays
- Adaptable to any 3D organoid culture-based drug screening platforms.

Sample Types

- Primary cell
- Primary tumor cell

Kit Contents

		B-P-00003-2、B-P-00003-4、B-P-00003-10 (Corresponding Amount and Content)	
Cat. No.	**Components**	**Amount**	**Content**
B-P-00003-A	A Gel (2X)	4、8、20	0.5mL / tube
B-P-00003-C	C Buffer (10X)	1、2、1	1*10ml、2*10ml、1*50 mL / BT
B-P-00003-D	D Buffer (10X)	1、2、1	1*10ml、2*10ml、1*50 mL / BT

Procedures

Preparation of the reagents

- **A Gel**: Place **A Gel** in 37℃ water bath for at least 10 min till completely thawed.
- **C Buffer (1X)**: Freshly dilute 10X **C buffe** with cold serum-free culture medium (e.g. serum-free DMEM) to 1X **C buffer** before use. (Do **NOT** dilute **C buffer** with PBS)
- **D Buffer (1X)**: Freshly dilute 10X **D buffer** with cold 1x PBS to 1X **D buffer** before use.

Preparation of 3D organoid culture

All steps are performed in laminar flow and the volume ratio of each component is added as indicated.

1. Place the **Thermal Conductive Sheet** on a ice pack. The sheet can rapidly and evenly cool down the culture plate.
2. Resuspend 1×10^5~10^7 cells in 1ml mixture of growth medium and **A Gel** at **1:1** ratio. For example, resuspend 1×10^5 cells in 500 μL growth medium and 500 μL **A Gel**.

   Note: Grow cells in appropriate media and culture conditions. Adherent cells should be cultured at ~80% confluency.
3. Place a culture plate/dish on **Thermal Conductive Sheet**/ice pack. Add 20-40 μL cell suspension mix from step 2 onto each well. The mixture should form gel after 5 min. **Note:** Test gel formation by gently touching gel with pipette tip, and gel thread should not be pulled out when retracting tips from gel surface.
4. Once gel has formed, add 1mL of cold 1X **C Buffer** to cover the dome for 15 min.
5. After 15 min incubation, **carefully** replace **C Buffer** with culture medium. Replace culture medium the following day.
6. Incubate cells at 37°C in CO_2 incubator for 7 to 14 days and observe spheroid formation with microscope. The Culture medium may be replaced every other day as required for proper growth of cells.

Dissolving 3D Gels for organoid collection

1. **Carefully** remove culture medium and wash the gel dome with 1X PBS.
2. **Carefully** remove 1X PBS and add 1 ml 1X **D buffer** onto gel for 5 min incubation at room temperature.
3. Gently pipet the solution with 1 mL tip until gel dome is completely dissolved.
4. Transfer the spheroid-contained solution to 1.5 mL tubes. Spin down the spheroids at 1,000 rpm for 10 min. Resuspend spheroids in media for use in

assay of interest.

Isolation of individual cells from spheroids

1. To isolate individual cells, isolate spheroids first with the procedure **Dissolving 3D Gels for spheroid collection** described above.
2. Add trypsin-EDTA to spheroids and incubate the solution at 37°C. Pipet the solution until spheroids dissociate completely.
3. When spheroids dissociate completely, add 3 volumes of 1X PBS and spin down cells with 10 min centrifugation at 1,000 rpm. Remove the supernatant and collect cells for proceeding assays.