

## 3DCultr Intestinal Cancer Organoid Growth Medium (Human)

### Product description

3DCultr Intestinal Cancer Organoid Growth Medium (Human) is a serum-free medium that can be used for the establishment and long-term culture of intestinal cancer organoids derived from cells or tissues. In the presence of extracellular matrix, The unique components and abundant cytokines contained in the culture medium can promote the rapid growth and formation of intestinal cancer organoids from intestinal cancer cells. The organoid formation process is smooth and rapid, while maintaining high characteristics and vitality of intestinal cancer cells, which provides a basis for subsequent Provide support for physiological functions, disease research and precision medicine of intestinal cancer organoids.

### Specifications

Catalog Number	C231106E/C231106S/C231106M
Specifications	50 mL/100 mL/500 mL

### Components

Component Number	Component Name	C231106E	C231106S	C231106M
C231106-A	Intestinal Cancer Organoid Growth Medium	45 mL	90 mL	450 mL
C231106-B	Nutritional components (10×)	5 mL	10 mL	50 mL

### Storage

stored at -25°C ~-15°C, the validity period is 1 year; when stored at 2~8°C, the validity period is 1 month.

### Notes

1. The operations such as packaging and use of the product should be carried out in a sterile environment, and the experimental equipment (such as: pipette tips, product tubes, etc.) in contact with the product should be pre-cooled before use.
2. For your safety and health, please wear a lab coat and disposable gloves.
3. For research use only.

## Instructions

### 1. Preparing human intestinal cancer organoid growth medium

Complete intestinal cancer organoid culture medium was prepared under sterile operating conditions. The following is the procedure for preparing 100 mL of complete culture medium. If the required amount is different, the amount can be adjusted accordingly.

- 1) Thaw component B at room temperature or slowly thaw at 2~8°C overnight. Avoid repeated freezing and thawing, prepare and thaw immediately;
- 2) Retrieve 90 mL of the basal growth medium(Component A) from the refrigerator and allow it to equilibrate to room temperature.
- 3) Add 10 mL of component B to the basic culture medium and mix evenly; if not used temporarily, store at 2~8°C for a short period of time.
- 4) You can add 1% double antibody when using.

### 2. Primary culture of human intestinal cancer

- 1) Specimen Collection: After the specimen is removed from the body, it should be collected promptly. Using sterile instruments to maintain aseptic conditions, place the tumor tissue in a 15 mL centrifuge tube containing 5 mL of primary tissue preservation solution, and transport at 4°C.
- 2) Washing: Remove the sample tube in a biosafety cabinet, discard the tissue preservation solution, and add an appropriate amount of cold PBS containing antibiotics. Repeat the washing process and then discard the PBS.
- 3) Repeat Washing: Repeat step 2 three times.
- 4) Tissue Processing: After removing the PBS buffer, transfer the tissue blocks to a sterile culture dish containing 10 mL of cold primary tissue preservation solution, and use sterile ophthalmic micro-scissors to chop the tissue into small pieces (approximately 0.5 mm~1 mm in diameter).
- 5) Repeat Washing: Use room temperature PBS, repeat step 2 three times.
- 6) Collect tissue fragments, add tissue digestion solution for digestion for 20~30 minutes, pipe repeatedly and pass through a 70  $\mu\text{m}$  mesh to collect intestinal cancer cells.If the cell yield is low, the process can be repeated once.
- 7) Red Blood Cell Lysis: Add 10 mL of red blood cell lysis buffer and shake on a rocker shaker at room temperature for 10 minutes.
- 8) Repeat Cleaning: After lysis is completed, use DMEM/F12 at room temperature and repeat step 2 three times.
- 9) Organoid Seeding Plate: Adjust the cell density to  $2\sim 3 \times 10^6$ , mix evenly with Matrix gel at a 1:1 ratio, seed the cell suspension in a 24-well plate at 40~60  $\mu\text{L}$  per well, and place it at 37°C for 15~30 min, then, add preheated organoid culture medium, 750  $\mu\text{L}$  per well.
- 10) Organoid Culture: Place the culture plate in a 37°C CO<sub>2</sub> incubator. Change the culture medium

every 2 days. When adding culture medium, keep the tip facing the side wall and add slowly.

11) Organoid Observation: Observe the organoids and take pictures every day to understand the initial number of organoids, proliferation rate, morphology, microbial contamination, etc.