TECHNICAL BULLETIN

Culturing Cancer-Derived Organoids Using IntestiCult™ Organoid Growth Medium (Human)

Cancer is one of the leading causes of death worldwide, with colorectal cancer representing one of the most common cancer types. Although cancer cell lines and animal models have revealed much information about intestinal cancers, cancer-derived intestinal organoids have been demonstrated to more faithfully recapitulate the tissue architecture, cellular heterogeneity, and morphology of the originating tumor.[1] Cancer-derived organoids are consequently proving to be useful experimental models for investigating cancer biology, including disease progression and the affected signaling pathways and niche requirements of the tumor.[2] Cancer-derived organoids have also enabled more translational applications, such as activating and expanding tumor-reactive T cell populations, predicting patient-specific treatment outcomes, and screening potential therapeutics.[3,4]

In this Technical Bulletin, we describe the culture of Wnt-independent cancer-derived organoids using IntestiCult™ Organoid Growth Medium (Human) (IntestiCult™ OGMH; Catalog #06010). For complete instructions, use this document in coordination with the IntestiCult™ Product Information Sheet (PIS; Document #DX21423), which includes a materials list and instructions for isolating human colonic crypts from biopsy samples, establishing human intestinal organoids from the isolated crypts, as well as expanding and maintaining organoid cultures via passaging.

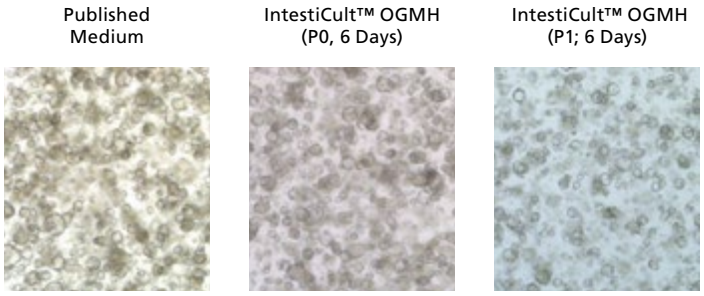

Figure 1. Growth of Cancer-Derived Organoids in IntestiCult™ OGMH.

Organoids were established from colorectal cancer biopsies in published medium,[1] then switched to IntestiCult™ OGMH (P0), as described below. Cancer-derived organoids demonstrated efficient growth both after establishment in IntestiCult™ OGMH, as well as after passaging. Data used with permission from Hubrecht Organoid Technology.

Protocol

The following protocol was developed in collaboration with Hubrecht Organoid Technology for the culture of Wnt-independent colorectal tumor biopsies in IntestiCult™ OGMH. Tumors that do not carry an activating mutation of the Wnt pathway cannot be maintained as organoids using this method.

A. Medium Preparation

The following example is for preparing 100 mL of IntestiCult™ OGMH for culture of Wnt-independent cancer-derived organoids (IntestiCult™ OGMH Component A + DMEM/F-12 with 15 mM HEPES; contact Customer Service to purchase IntestiCult™ OGMH Component A only). If preparing other volumes, adjust accordingly.

1. Thaw IntestiCult™ OGMH Component A at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly. Use immediately or aliquot and store at -20°C for up to 3 months. After thawing aliquots, use immediately. Do not re-freeze.
2. Add 50 mL IntestiCult™ OGMH Component A to 50 mL DMEM/F-12 with 15 mM HEPES. Mix thoroughly. If not used immediately, store at 2 - 8°C for up to 1 week.
3. Add desired antibiotics immediately before use (e.g. 50 µg/mL gentamicin).

B. Isolation of Tissue from Tumor Biopsies

1. Thaw 100 µL of Matrigel® on ice.
   NOTE: This is sufficient for plating up to 4 culture domes.
2. Place the following reagents on ice: D-PBS (Without Ca++ and Mg++) and DMEM + 1% BSA (See PIS [Document #DX21423] for preparation).
3. Warm a tissue culture-treated 24-well plate in a 37°C incubator for at least 2 hours.
4. In a 15 mL conical tube, wash the tissue sample with 10 mL of ice-cold PBS. Allow the tissue to settle by gravity (~5 seconds) then aspirate the supernatant.
5. Repeat step 4, leaving 1 mL of supernatant in the tube.
6. Transfer the tissue and remaining supernatant to a 1.5 mL microcentrifuge tube using a 1 mL pipettor.
7. Using sterile scissors, thoroughly mince the tissue into ~5 mm pieces. Transfer the tissue fragments to a new 15 mL conical tube using a 1 mL pipettor. Rinse the microcentrifuge tube with PBS and add the rinse to the tissue fragments.
8. Allow the tissue fragments to settle by gravity (~5 seconds) then aspirate the supernatant.
9. Add 10 mL of Gentle Cell Dissociation Reagent. Incubate at 37°C on a rocking platform set at medium speed (~40 rpm) for 60 minutes.

10. Centrifuge at 290 *x g* for 5 minutes. Aspirate the supernatant.
    NOTE: For the remainder of the protocol, pre-wet pipette tips with DMEM + 1% BSA before manipulating the tissue sample. This prevents crypts from sticking to the wall of the pipette tip.
11. Add 1 mL of ice-cold DMEM + 1% BSA. Vigorously pipette up and down 20 times with a 1 mL pipettor.
    NOTE: Avoid touching the tube with the pipette tip.
12. Using a 1 mL pipettor, pass the contents of the tube through a 70 µm cell strainer tilted on its side into a new 15 mL conical tube. Rinse the original tube with 1 mL of DMEM + 1% BSA and pass through the strainer into the tube.

C. Organoid Culture From Isolated Biopsy Tissue

For complete instructions on plating isolated tissue as organoid cultures, proceed to section B, step 2 in the PIS (Document #DX21423). When plating organoids derived from cancerous tissue, use the medium prepared in section A (above) instead of complete IntestiCult™ OGMH.

Growth medium should be replaced every 2 days, and cultures can be passaged every 6 - 12 days. For complete passaging instructions, refer to section C in the PIS (Document #DX21423). When passaging organoids derived from cancerous tissue, use the medium prepared in section A (above) instead of complete IntestiCult™ OGMH.

Product Information

PRODUCT	CATALOG #
IntestiCult™ Organoid Growth Medium (Human)	06010
DMEM/F-12 with 15 mM HEPES	36254
Corning® Matrigel® Matrix, Growth Factor Reduced (GFR), Phenol Red-Free	Corning® 356231
Gentle Cell Dissociation Reagent	07174
D-PBS (Without Ca++ and Mg++)	37350
CryoStor® CS10	07930
70 µm Reversible Strainer, Small	27216
Costar® 24-Well Flat-Bottom Plate, Tissue Culture-Treated	38017

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SCIENTIFIC RESOURCES

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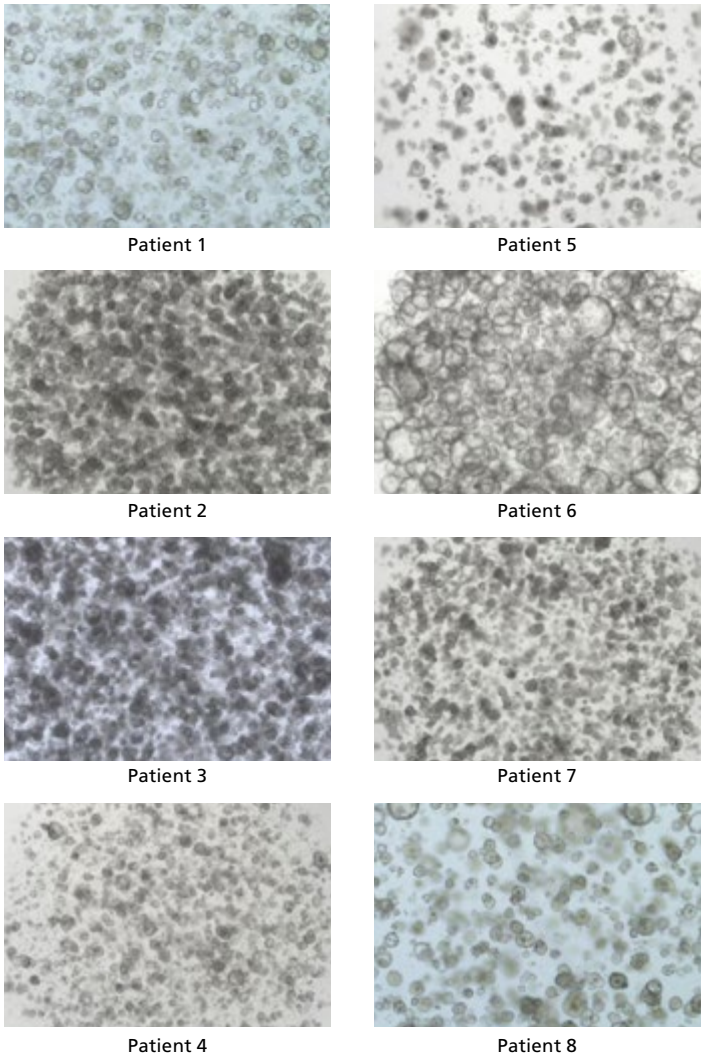

Figure 2. IntestiCult™ Organoid Growth Medium (Human) Enables Organoid Growth Across Different Patients

Organoids were established in published medium[1] from colorectal tumor biopsy samples, then switched to IntestiCult™ OGMH after passaging as described above. Organoids were passaged twice in IntestiCult™ and imaged at the end of the second passage (day 6 - 12). Data used with permission from Hubrecht Organoid Technology.

References

1. Sato T et al. (2011) Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology. 141(5): 1762–72.
2. Fujii M et al. (2016) A colorectal tumor organoid library demonstrates progressive loss of niche factor requirements during tumorigenesis. Cell Stem Cell. 18(6): 827–38.
3. Dijkstra K et al. (2018) Generation of tumor-reactive T cells by co-culture of peripheral blood lymphocytes and tumor organoids. Cell. 174(6): 1586–98.
4. Vlachogiannis G et al. (2018) Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. Science. 359(6378): 920–26.