MasterAim® Bile Duct Cancer Organoid Complete Medium

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Comprehensive Solution for Organoid Culture

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Overview

The MasterAim® Bile Duct Cancer Organoid Complete Medium effectively replicates the in *vivo* microenvironment of tumor cells, streamlining the establishment and maintenance of human bile duct cancer organoids. Patient-derived organoids cultured with the complete medium faithfully maintain the morphological, genomical, and pathophysiological characteristics of their corresponding *in vivo* tumors. They also demonstrate comparable pharmacological profiles and treatment response.

MasterAim®Bile Duct Cancer Organoid Complete Medium comprises a complete set of reagents for establishment and maintenance of organoids, with optimized protocol and improved efficiency of organoid culture. It additionally offers the flexibility to scale up for creating patient-relevant models and facilitating high-throughput screening.

Product Information

Research use only, the following components are sold as a complete kit (Catalog #10-100-254).

COMPONENT NAME	COMPONENT #	SIZE	STORAGE&SHELF LIFE
MasterAim® Bile Duct Cancer Organoid Basal Medium	100-255	95 mL	-20°C, 12 months; 4°C, 3 months
MasterAim® Bile Duct Cancer Organoid Supplement	100-256	5 mL	-20°C, 12 months

Reagents Required But Not Included:

PRODUCT NAME	PRODUCT #	SIZE	STORAGE&SHELF LIFE
MasterAim® Tissue Preservation Medium	100-032	100 mL	--20°C, 12 months; 4°C, 2 weeks
MasterAim® Tissue Dissociation Medium I	100-047	100 mL	-20°C, 12 months; 4°C, 2 weeks
MasterAim® Tissue Dissociation Medium II	100-048	50 mL	-20°C, 12 months; 4°C, 2 weeks
MasterAim® Organoid Cryopreservation Medium	100-045	100 mL	4°C, 12 months
MasterAim® Primary Enhancer	100-008	0.5 mL	-20°C, 12 months
MasterAim® Anti-Adherence Solution	100-291	100 mL	4°C, 12 months
DPBS Balanced Salt Solution (without Calcium and Magnesium Ions, without Phenol Red)	100-184	500 mL	AMB，24 months
TrypLE	/	/	/
RBC Lysis Solution	/	/	/
Matrigel	/	/	/

Directions for Use

Please read the entire protocol before proceeding.

Use sterile technique when performing the following protocols.

Preservation of Primary Tissue

1. Pre-chill Tissue Preservation Medium to 2-8 °C before use.

2. Transfer fresh tissue directly into the pre-chilled **MasterAim® Tissue Preservation Medium**（**#100-032**）. If the tissue has already been placed in other buffers or media, replace the complete liquid with the pre-chilled Tissue Preservation Medium. The tissue must be completely covered with the Tissue Preservation Medium. Store or ship sample at 2-8 °C until the start of the isolation. Process the tissue within 48 h from collection.

Preparation of MasterAim® Bile Duct Cancer Organoid Complete Medium

1. Preparation of Reagents: Use sterile technique to prepare the bile duct cancer organoid complete medium. Thaw **MasterAim® Bile Duct Cancer Organoid Basal Medium**（**#100-255**）and **MasterAim® Bile Duct Cancer Organoid Supplement**（**#100-256**）on ice.

2. Preparation of Complete Medium: Mix MasterAim® Bile Duct Cancer Organoid Basal Medium and MasterAim® Bile Duct Cancer Organoid Supplement thoroughly.

3. For Establishment of Primary Organoids Culture: Take 10 mL of Bile Duct Cancer Organoid Complete Medium, and transfer 100 µL **MasterAim® Primary Enhancer**（**#100-008**）to 10 mL Bile Duct Cancer Organoid Complete Medium. Mix thoroughly.

4. For Maintenance of Organoids: No need to add MasterAim® Primary Enhancer.

NOTE:

MasterAim® Bile Duct Cancer Organoid Complete Medium is formulated without antibiotic or antifungal components. Supplement with these ingredients at standard concentrations prior to use. Recommended concentration is 1%.

If not used immediately, aliquot reagent (s) and store at -20°C for no more than 3 months. Do not exceed the shelf life of the reagent (s). After thawing, use immediately. Do not re-freeze.

Establishment of Organoids from Primary Tissue

1. Process the Tissue: Transfer fresh tissue and cold **DPBS-PS**（**#100-184**）into 10 cm petri dish on ice. Assess the obtained tissue, If fat or muscle tissues are present, remove these non-epithelial components as much as possible using surgical scissors or scalpels and forceps.

2. Rinse the Tissue: Rinse the tissue with DPBS-PS until the supernatant is clear. Remove and discard the supernatant.

3. Preparation of digestion: Add 200 µL **MasterAim® Tissue Dissociation Medium I**（**#100-047**）to rinse the tissue in a 10 cm petri dish. Use scissors to cut tissue into small pieces (0.5 - 1 mm3), and transfer tissue pieces into a 15 mL conical tube rinsed with **MasterAim® Anti-Adherence Solution (#100-291)** on ice.

4. Digestion Ⅰ: Add 4 mL MasterAim® Tissue Dissociation Medium I to conical tube. Incubate at 37°C, with shaking for 30 minutes to 40 minutes.

Carefully assess the digestion process under the microscope every 15 min to prevent over-digestion. Terminate Digestion I when most cell clusters are released from the primary tissue.

5. Termination of Digestion Ⅰ: Add 8 mL DPBS-PS to conical tube, centrifuge at 300 x g for 5 minutes. Aspirate and discard as much of the supernatant as possible without disturbing the pellet.

6. DigestionⅡ: Add 2 mL **MasterAim® Tissue Dissociation Medium Ⅱ (#100-048)** to conical tube. Incubate at 37°C, with shaking for 10 minutes to 15 minutes. Carefully assess the digestion process under the microscope, and terminate the digestionⅡ when the diameter of most cell clusters is less than 200 μm.

7. Termination of Digestion Ⅱ: Add 8 mL DPBS-PS to conical tube, centrifuge at 300 x g for 5 minutes. Aspirate and discard as much of the supernatant as possible without disturbing the pellet.

8. Cell Collection: Add 10 mL of DPBS-PS to the conical tube to resuspend pellet, and filter the mixture using a 100 μm cell strainer rinsed with MasterAim® Anti-Adherence Solution. Collect the filtered cells and centrifuge at 300 x g for 5 minutes. Aspirate and discard as much of the supernatant as possible without disturbing the pellet.

9.Optional: In case of a visible red pellet, aspirate the supernatant, and resuspend the pellet using RBC Lysis Solution to lyse the erythrocytes. Refer to the product manual for the specific experimental procedures. Centrifuge at 300 x g for 5 minutes, aspirate, and discard as much of the supernatant as possible without disturbing the pellet. Resuspend the pellet using 1 mL of DPBS-PS.

10. Cell Counting: Counting the number of live cells using a cell counter or hemocytometer. Centrifuge at 300 x g for 5 minutes, aspirate, and discard as much of the supernatant as possible without disturbing the pellet.

11. Dome Culture Initiation: Resuspend the organoids in Matrigel, keeping Matrigel on ice to prevent it from solidifying. Plate the Matrigel-containing organoids at the bottom of 24-well cell culture plates in droplets of 50 μL each around the center of each well. The number of domes that can be seeded will depend on the size of the original tissue sample and the live cell count. At least 2,000 live cells should be seeded per μL, but higher seeding densities are recommended if possible. Place the lid on the culture plate, carefully place the plate in cell culture incubator at 37°C and 5% CO2 for 5 minutes, inverted the plate in cell culture incubator at 37°C and 5% CO2 for 25 minutes to facilitate domes formation.

12. Liquid Adding: Remove the plate from the incubator and place in the biosafety cabinet. Without disturbing the domes, carefully add 600 μL of 37°C MasterAim® organoid complete medium against the side of each well containing a dome. Do not pipette directly onto the domes. Add 1 mL sterile DPBS-PS to any empty wells to maintain humidity.

13. Organoid Culture: Place the lid on the culture plate, carefully place the plate in cell culture incubator at 37°C and 5% CO2. Perform a full-medium change every 2 - 3 days by aspirating the medium and adding fresh complete medium at room temperature. Usually, visible organoid could be detected under the microscope 3 - 4 days after inoculation.

Passaging of Organoids

1. Passaging Standard: Before passaging, the diameter of most organoids is greater than 100 μm.

2. Digestion: Remove the plate from the incubator and place it in the biosafety cabinet. Without touching the dome, aspirate and discard the medium in each well to be passaged. Using a 1 mL pipettor, forcefully add 0.5 mL of TrypLE to the center of each dome and pipette up and

down to disrupt the Matrigel. Incubate the plate at 37°C for 5 minutes to 8 minutes. Add 1 mL of DPBS-PS to t terminate the digestion when the diameter of most organoids is between 40 - 60 µm.

3. Organoids Collection: Transfer mixture to conical tube, centrifuge at 300 x g for 5 minutes, aspirate and discard as much of supernatant as possible without disturbing the pellet. Resuspend the pellet using 1 mL of DPBS-PS.

4. Cell Counting: Counting the number of live cells using a cell counter or hemocytometer. Centrifuge at 300 x g for 5 minutes, aspirate and discard as much of the supernatant as possible without disturbing the pellet.

5. Dome Culture Initiation: Resuspend the organoids in Matrigel, keeping Matrigel on ice to prevent it from solidifying. Plate the Matrigel-containing organoids at the bottom of 24-well cell culture plates in droplets of 50 µL each around the center of each well. At least 200 cell clusters should be seeded per dome, but higher seeding densities are recommended if possible. Place the lid on the culture plate, carefully place the plate in cell culture incubator at 37°C and 5% CO2 for 5 minutes, inverted the plate in an incubator at 37°C and 5% CO2 for 25 minutes to facilitate domes formation.

6. Liquid adding: Remove the plate from the incubator and place in the biosafety cabinet. Without disturbing the domes, carefully add 600 µL of 37°C MasterAim® organoid complete medium against the side of each well containing a dome. Do not pipette directly onto the domes. Add 1 mL sterile DPBS-PS to any empty wells to maintain humidity.

7. Organoid Culture: Place the lid on the culture plate, carefully place the plate in an incubator at 37°C and 5% CO2. Carefully assess the culture process under the microscope, perform a full-medium change every 2 - 4 days by aspirating the medium and adding fresh complete medium at room temperature until they are passaged, and observe culture status of organoids under microscope.

Cryopreservation of Organoids

1. Cryopreservation Standard: Before cryopreservation, the diameter of most organoids is greater than 100 µm.

2.Digestion: Remove the plate from the incubator and place it in the biosafety cabinet. Without touching the dome, aspirate and discard the medium in each well to be passaged. Using a 1 mL pipettor, forcefully add 0.5 mL of TrypLE to the center of each dome and pipette up and down to disrupt the Matrigel. Incubate the plate at 37°C for 3 minutes. Add 1 mL of DPBS-PS to terminate the digestion when the diameter of most organoids is between 40 - 60 µm.

3. Organoids Collection: Transfer suspension to conical tube, centrifuge at 300 x g for 5 minutes, aspirate and discard as much of the supernatant as possible without disturbing the pellet. Resuspend the pellet using 3 mL of DPBS-PS, centrifuge at 300 x g for 5 minutes, aspirate and discard as much of the supernatant as possible without disturbing the pellet. Resuspend the pellet using 1 mL of DPBS-PS.

4. Cell Counting: Counting the number of live cells using a cell counter or hemocytometer. Centrifuge at 300 x g for 5 minutes, aspirate and discard as much of the supernatant as possible without disturbing the pellet.

5. Cryopreservation: Resuspend the organoids with appropriate amount of **MasterAim® Organoid Cryopreservation Medium** (**#100-045**) according to the densities of organoids, mix thoroughly. Pellets can be divided into multiple cryovials based on the desired density (500,000 cells per 1 mL cryovial). Immediately transfer 1 mL of suspension into a pre-cooled cryovial and transfer into a freezing container. Repeat for remaining cryovials. Immediately transfer freezing container to -80°C. After 24 hours, transfer vials from -80°C into the gas phase of a liquid nitrogen tank for long-term storage.