bioGenous™ Neuroendocrine Neoplasm Organoid Kit

Catalog: K2117-NN

Product Description:

bioGenous™ Neuroendocrine Neoplasm Organoid Kit is a chemically defined cell culture medium for the human neuroendocrine neoplasm organoids. Patient-derived cancer organoids recapitulate the genomic and pathological features of original tumors and therefore hold great promise for medical research and precision medicine.

Product Information:

Component	Catalog#	Volume	Storage & Stability
bioGenous™ Neuroendocrine Neoplasm Organoid Basal Medium	K2117-NN-A100/A500	100mL/500mL	2-8℃，12 months
bioGenous™ Neuroendocrine Neoplasm Organoid Supplement B (50x)	K2117-NN-B100/B500	2mL/10mL	-20℃, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Neuroendocrine Neoplasm Organoid Supplement C (250x)	K2117-NN-C100/C500	0.4mL/2mL	-20℃, avoid repeated freeze-thaw cycles, 12 months

Materials & Reagents Required But Not Included:

Manufacturer	Materials	Catalog#
bioGenous™	Primary Tissue Storage Solution	K601005
bioGenous™	Cancer Organoid Basal Medium	B213152
bioGenous™	Tumor Tissue Digestion Solution	K601003
bioGenous™	Red Blood Cell Lysis Solution	E238010
bioGenous™	Anti-Adherence Rinsing Kit	E238002
bioGenous™	Organoid Cryopreservation Medium(Serum Free)	E238023
bioGenous™	Organoid Dissociation Solution	E238001
bioGenous™	Organoid Culture ECM(Reduced Growth Factor)	M315066
	Fetal Bovine Serum (FBS)	-
	DPBS (1X), liquid, contains no calcium or magnesium	-
	100 μm Cell Strainer	-

Preparation of Neuroendocrine Neoplasm Organoid Complete Medium

Use a sterile technique to prepare the neuroendocrine neoplasm organoid complete medium. The following example is for preparing 10mL complete medium. If preparing other volumes, adjust accordingly.

1. Thaw Neuroendocrine Neoplasm Organoid Supplement B (50x) and Neuroendocrine Neoplasm Organoid Supplement C (250x) on ice. Mix thoroughly.
   NOTE: Once thawed, use immediately or aliquot and store at -20°C for not more than 10 months. After thawing the aliquots, use immediately. Do not re-freeze.
2. Add 200μL Neuroendocrine Neoplasm Organoid Supplement B (50x) and 40μL Neuroendocrine Neoplasm Organoid Supplement C (250x) to 9.76mL Neuroendocrine Neoplasm Organoid Basal Medium. Mix thoroughly.
   NOTE: If not used immediately, store the complete medium at 2-8°C for not more than 2 weeks. The Neuroendocrine Neoplasm Organoid Supplement B contains fungicides and antibiotics (50x).

Protocol for Establishment of Patient-Derived Neuroendocrine Neoplasm Organoids

CAUTION *Studies involving primary human tissue material must follow all relevant institutional and government regulations. Informed consent must be obtained from all subjects before the collection of the primary human tissue material.*

Establishment of Organoids from Primary Tissue

1. Collect primary human neuroendocrine neoplasm tissue pieces in ice-cold Primary Tissue Storage Solution (K601005) with conical tubes. Keep tissue samples at 4°C until the start of the isolation.
2. Assess whether the obtained tissue pieces consist purely of epithelium. If fat or muscle tissues are present, remove these non-epithelial components as much as possible using surgical scissors or scalpels and forceps under a dissection microscope. If no fat or muscle tissues are present, continue to the next step immediately.
3. Rinse the tissue with Cancer Organoid Basal Medium (B213152) or DPBS twice.
4. Mince the tissue into small fragments of 1-3 mm^3 in a cell culture dish using surgical scissors or scalpels.

5. Digest the tissue fragments with 10mL of Tumor Tissue Digestion Solution (K601003) in a 15mL conical tube at 37°C, with variable incubation times ranging from 10 min to 30 min. Carefully monitor the digestion process, mixing the content of the tube every 5-10 min by shaking vigorously or pipetting the mixture up and down. The digestion process could be finished when most of the tissue fragments are able to pass through the 1mL pipette tips.
6. Add FBS to the tissue digestion mixture to a final concentration of 2%, and filter using a 100 μm cell strainer.
7. Collect and centrifuge the filtered cells at 250g for 3 min at 4 °C. In case of a visible red pellet, aspirate the supernatant, and resuspend the pellet using 2mL of Red Blood Cell Lysis Solution (E238010) to lyse the erythrocytes at room temperature for 1 min and centrifuge at 250g for 3 min at 4°C.
8. Aspirate the supernatant and resuspend the pellet in Cancer Organoid Basal Medium, centrifuge at 250g for 3 min at 4°C, and repeat this step once more time.
9. Aspirate the supernatant and resuspend the pellet in bioGenous™ Organoid Culture ECM (M315066). The ECM should be kept on ice to prevent it from solidifying. Perform the process as quickly as possible. The amount of ECM used depends on the size of the pellet. Approximately 10,000 cells should be plated in 25 μL of ECM.
   CRITICAL: Do not overly dilute the ECM (ECM should be >70% (ECM vol/Total vol)), as this may inhibit the proper formation of the solid droplets.
10. Plate the ECM containing organoids at the bottom of 24-well cell culture plates in droplets of ~30 μL each around the center of the well.
    CRITICAL: Once the organoids are resuspended in ECM, proceed with plating as quickly as possible, as the ECM may solidify in the tube or pipette tip. Do not let the ECM touch the wall of the wells.
11. Place the culture plate into a humidified incubator at 37 °C and 5% (vol/vol) CO_2 for 15-25 min to let the ECM solidify.
12. Prepare the required amount of neuroendocrine neoplasm organoid complete medium.
13. Once the ECM droplets have solidified (15-25 min), open the plate and carefully add 500 μL of organoid complete medium to each well.
    CRITICAL: Do not add the medium directly on top of the ECM droplets, as this might disrupt the droplets.
14. Place the culture plate in a humidified incubator at 37 °C and 5% (vol/vol) CO_2.
15. Change the medium every 3-4 d by carefully aspirating the medium from the wells and replacing it with a fresh, pre-warmed organoid complete medium.
16. Closely monitor organoid formation. Ideally, patient-derived neuroendocrine neoplasm organoids should be passaged for the first time between 7 and 10 d after the initial plating.Examples of successful culture in primary, passage and resuscitation of human neuroendocrine neoplasm organoids are shown in Figure 1.

Splitting and Passaging of Organoids

17. Pipette up and down to disrupt the ECM and transfer the organoid suspension to a 1.5 mL conical tube.
18. Pipette the organoid suspension up and down to mix thoroughly by pipetting against the bottom of the tube to create pressure, which will aid the removal of ECM.
19. Centrifuge organoids at 250g for 3 min at room temperature.
20. Aspirate the supernatant and split organoids using either Organoid Dissociation Solution (E238001) or by mechanical disruption. For Organoid Dissociation Solution-based cell dissociation, resuspend the pellet in 0.2 mL of Organoid Dissociation Solution, pipette up and down and incubate at 37 °C until organoids fall apart. Pipette up and down with a filter tip for ≥8 times every 2 min to aid in the disruption of the organoids. Closely monitor the digestion to keep the incubation time in the Organoid Dissociation Solution to a minimum. In case of mechanical disruption, resuspend the pellet in 1.5 mL of Cancer Organoid Basal Medium. Carefully pipette the organoid suspension up and down 30 times by pipetting against the bottom of the tube to create pressure, which will aid organoid disruption.
    CRITICAL: Do not dissociate in Organoid Dissociation Solution for >5 min, as this may result in poor organoid outgrowth or even loss of the culture. As a rule of thumb, digestion is complete if a mixture of small lumps of cells (consisting of 10–50 cells) can be observed.
21. After shearing is complete, wash once by topping up with 1 mL of Cancer Organoid Basal Medium, and centrifuge at 250g for 3 min at room temperature.
22. Aspirate the supernatant and resuspend the organoid pellet in 70% (vol/vol) ECM, and plate organoids in droplets at the bottom of a culture plate as described in Step 10. After plating is complete, transfer the plate into a humidified incubator at 37 °C and 5% (vol/vol) CO_2 for 15–25 min.
23. Pre-warm neuroendocrine neoplasm organoid complete medium at 37 °C.
24. After the ECM droplets have solidified (15–25 min), carefully pipette the pre-warmed medium into the wells.
25. Place culture plates in a humidified incubator at 37 °C and 5% (vol/vol) CO_2 until the organoids are needed for further experiments.

Appendix 1. Examples of different generations of human neuroendocrine neoplasm organoids.

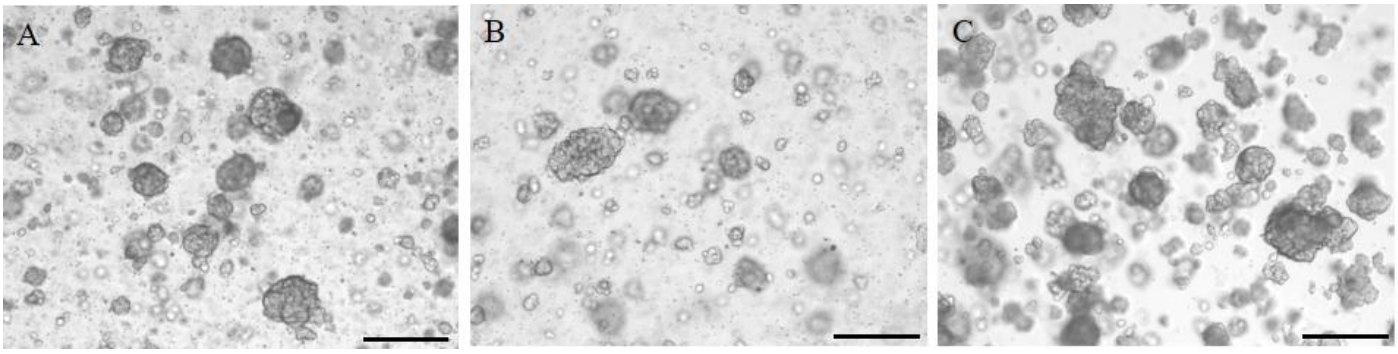

Figure 1. Examples of successful culture in primary, passage and resuscitation of human neuroendocrine neoplasm organoids. (A) The growth status of neuroendocrine neoplasm organoids in primary culture (P0). The organoids are irregular folds with smooth edges and high dioptering,and the diameter is about 80-100 μm. (B) The growth status of neuroendocrine neoplasm organoids in the first passage culture(P1), passage organoids mainly presented parenchymal shapes. (C) The resuscitated cultured organoids showed a steady growth trend. (scale bar: 200 μm).

Last updated on 20th July 2023