bioGenous™ Human Pancreas Organoid Kit

Catalog: K2013-PD

Product Description:

bioGenous™ Human Pancreas Organoid Kit is a chemically defined cell culture medium for human pancreas ductal organoids. This culture medium provides with the essential nutrients needed to self-renewal and self-organization while retaining the ability their original pancreatic duct function. Pancreas organoids display all hallmarks of the ductal epithelium in terms of architecture, cell type composition, and self-renewal dynamics. The significant expression of biomarkers of pancreatic stem cells, ductal cells, and progenitor cells indicating the retention of the original pancreas properties. Pancreas organoids hold great promise for unprecedented studies of human pancreas duct regeneration and disease modelling.

Product Information:

Component	Cat#	Volume	Storage & Stability
bioGenous™ Human Pancreas Organoid Basal Medium	K2013-PD-A100/A500	100mL/500mL	2-8℃，12 months
bioGenous™ Human Pancreas Organoid Supplement B (50x)	K2013-PD-B100/B500	2mL/10mL	-20℃, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Human Pancreas Organoid Supplement C (250x)	K2013-PD-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™	Epithelial Organoid Basal Medium	B213151
bioGenous™	Organoid Dissociation Solution	E238001
bioGenous™	Tissue Digestion Solution	K601008
bioGenous™	Anti-Adherence Rinsing Kit	E238002
bioGenous™	Organoid Cryopreservation Medium （Serum Free)	E238023
bioGenous™	Organoid Culture ECM (Reduced Growth Factor)	M315066
	DPBS (1X), liquid, contains no calcium or magnesium	-
	Fetal Bovine Serum (FBS)	-

Preparation of Human Pancreas Organoid Complete Medium

Use a sterile technique to reconstitute the human pancreas organoid complete medium. The following example is for preparing a 10 mL Medium. If preparing other volumes, adjust accordingly.

1. Thaw Human Pancreas Organoid Supplement B (50x), and Human Pancreas 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 them immediately. Do not re-freeze.
2. Add 200 µL Human Pancreas Organoid Supplement B (50x), and 40 µL Human Pancreas Organoid Supplement C (250x) to the Human Pancreas Organoid Basal Medium. Mix thoroughly.
   NOTE: If not used immediately, store the complete medium at 2-8°C for not more than 2 weeks. bioGenous™ Human Pancreas Organoid Supplement B contains fungicides and antibiotics (50x).

Protocol for Establishing Human Pancreas Organoids

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

Establishment of Pancreas Organoids from Primary Tissue

1. Collect primary human pancreas tissues immediately after surgical excision in an ice-cold Primary Tissue Storage Solution (K601005) in conical tubes at 4°C. Keep on ice until ready for processing.
2. Assess whether the obtained tissue pieces consist purely of epithelium or if they also contain fat or muscle tissues. As much as possible, remove all non-epithelial components using surgical scissors, scalpels, and forceps under a dissection microscope. If no fat or muscle tissues is present, continue to the next step immediately.
3. Rinse the liver tissue with Epithelial Organoid Basal Medium(B213151) or DPBS until the supernatant is clear.
4. Before ducts isolation, thaw bioGenous™ Organoid Culture ECM on ice and keep it cold. Add 5 mL of FBS to 45 mL of Epithelial Organoid Basal Medium to prepare 10% (vol/vol) FBS medium.
5. Mince the tissue into small fragments of 5 mm^3 in a cell culture dish using surgical scissors or scalpels.
   CRITICAL The dissected samples must be small enough to pass through the tip of a 10 mL pipette.

6. Place the dissected pieces of sample into a 15 mL conical tube containing 10 mL of cold Epithelial Organoid Basal Medium with 1% FBS.
7. Wash the samples by pipetting up and down with a 10 mL pipette at least ten times.
   CRITICAL For the subsequent steps, coat the inner surface of pipette tips with bioGenous™ Anti-Adherence Rinsing Solution (E238002) before use to avoid the adherence of the samples on the pipette wall.
8. Stand the tube still until the samples settle at the bottom. Aspirate the supernatant with a 10 mL pipette and add 10 mL of pre-warmed Tissue Digestion Solution (K601008).
9. Digest the tissue fractions at 37℃ with rotation at the speed of 100 rpm. The digestion time should not exceed 30 mins.
   CRITICAL To prevent over-digestion, one should examine under the microscope if the duct structure appears during digestion.
10. Once the duct structure appears, stop digestion by the addition of FBS to a final concentration of 2% and pipette gently up and down.
11. Stand the tube for 1-2 min. Transfer the supernatant into a new tube.
12. Add 10mL Epithelial Organoid Basal Medium and repeat Step 11 one more time.
13. Spin the supernatant at 300g for 3 min at 4°C. Aspirate and discard the supernatant.
14. Re-suspend the pellet with 10mL Epithelial Organoid Basal Medium and spin at 300g for 3 min at 4°C.
15. Repeat Step 14 twice.
16. Aspirate the supernatant and resuspend the pellet in ECM. ECM should be kept on ice to prevent it from solidifying; thus, work quickly. The amount of ECM depends on the size of the pellet. Approximately 20-100 ducts should be plated in 25 µL of ECM.
    CRITICAL Do not dilute ECM too much (ECM should be >70% (ECM vol/Total vol)) to ensure the formation of solid droplets.
17. Plate the ECM containing organoids on 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.
18. 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.
19. Prepare the required amount of bioGenous™ Human Pancreas Organoid Medium.
20. Once the ECM droplets are 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.
21. Place the culture plate in a humidified incubator at 37 °C and 5% (vol/vol) CO_2.
22. Change the medium every 3 days by carefully aspirating the medium from the wells and replacing it with fresh, pre-warmed Human Pancreas Organoid Medium.
23. Closely monitor organoid formation. Ideally, human Pancreas organoids should be passaged for the first time between 5 and 8 days after initial plating.

Passaging of Human Pancreas Organoids

24. Pipette the culture medium up and down to disrupt the ECM and transfer the organoid suspension into a 1.5 mL conical tube.
25. Pipette the organoid suspension up and down to mix thoroughly. Use the bottom of the tube to create pressure to aid the removal of ECM.
26. Centrifuge the organoid suspension at 300g for 3 min at room temperature.
27. Aspirate the supernatant and split the organoids by mechanical disruption or using Organoid Dissociation Solution (E238001). For mechanical disruption, resuspend the pellet in 1 mL of Epithelial Organoid Basal Medium. Pipette the organoid suspension up and down 30 times. Use the bottom of the tube to create pressure to aid in organoid disruption. In the case of using Organoid Dissociation Solution, 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 ≥10 times every 1 min to aid in the disruption of the organoids. Monitor the digestion closely to keep the incubation time in Organoid Dissociation Solution to a minimum.
    CRITICAL Do not dissociate in Organoid Dissociation Solution for >3 mins, 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.
28. After completely disrupting the organoids, wash once by adding 1 mL of Epithelial Organoid Basal Medium and centrifuge at 200g for 3 min at room temperature.
29. Aspirate the supernatant and resuspend the organoid pellet in 70% (vol/vol) ECM and plate organoids in droplets on the bottom centre of a culture plate as described in Steps 12. After plating is complete, transfer the plate into a humidified incubator at 37 °C and 5% (vol/vol) CO_2 for 15–20 mins.
30. Pre-warm Human Pancreas Ductal Organoid Complete Medium at 37 °C.
31. After the ECM droplets have solidified (15–20 min), carefully pipette the pre-warmed medium into the wells.

32. Incubate culture plates in a humidified incubator at 37 °C and 5% (vol/vol) CO_2 until the organoids are needed for further experiments.

Last updated on 20th March 2023