bioGenous™ Human Gastric Epithelial Organoid Kit

Catalog: K2004-HG

Product Description:

bioGenous™ Human Gastric Epithelial Organoid Kit is a chemically defined cell culture medium for establishment and maintenance of human gastric epithelial organoids derived from adult stem cells. Self-renewal of the gastric epithelium is driven by the proliferation of stem cells and their progenitors. Human gastric epithelial organoids display all hallmarks of the gastric epithelium in terms of architecture, cell type composition, and self-renewal dynamics, therefore hold great promise for unprecedented studies of human gastric epithelial homeostasis and disease.

Product Information:

Component	Component Cat#	Volume	Storage& Stability
bioGenous™ Human Gastric Epithelial Organoid Basal Medium	K2004-HG-A100/A500	100 mL/500 mL	4℃，12 months
bioGenous™ Human Gastric Epithelial Organoid Supplement B(50x)	K2004-HG-B100/B500	2 mL/10 mL	-20℃, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Human Gastric Epithelial Organoid Supplement C(250x)	K2004-HG-C100/C500	0.4 mL/2 mL	-20℃, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Human Gastric Epithelial Organoid Supplement D(250x)	K2004-HG-D100/D500	0.4 mL/2 mL	-20℃, avoid repeated freeze-thaw cycles, 12 months

Materials & Reagents Required But Not Included:

Vender	Materials	Catalog#
bioGenous™	Primary Tissue Storage Solution	K601005
bioGenous™	Epithelial Organoid Basal Medium	B213151
bioGenous™	EDTA	E219121
bioGenous™	Organoid Dissociation Solution	E238001
bioGenous™	Anti-Adherence Rinsing Solution	E238002
bioGenous™	Organoid Cryopreservation Medium （Serum Free）	E238023
Corning®	Matrigel®Growth Factor Reduced Basement Membrane Matrix	356231
	Fetal Bovine Serum (FBS)	-
	DPBS (1X), liquid, contains no calcium or magnesium	-

Preparation of Human Gastric Epithelial Organoid Primary Culture Medium and Maintenance Medium

Use sterile technique to prepare the Human Gastric Epithelial Organoid Primary Culture Medium and Maintenance Medium. Gastric epithelial organoids grown in Human Gastric Epithelial Organoid Maintenance Medium contain LGR5+ stem cells, pit mucous cells, gland mucous cells, chief cells, as well as a low number of enteroendocrine cells. The following examples are for preparing 10 mL of Primary Culture Medium and Maintenance Medium. If preparing other volumes, adjust accordingly.

1. Thaw Human Gastric Epithelial Organoid Supplement B(50x), Human Gastric Epithelial Organoid Supplement C(250x) and Human Gastric Epithelial Organoid Supplement D(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. For Human Gastric Epithelial Organoid Primary Culture Medium which used specifically for primary culture and resuscitation. Add 200 µL Human Gastric Epithelial Organoid Supplement B(50x), 40 µL Human Gastric Epithelial Organoid Supplement C(250x) and 40 µL Human Gastric Epithelial Organoid Supplement D(250x) to 9.72 mL Human Gastric Epithelial Organoid Basal Medium. Mix thoroughly.
3. For Human Gastric Epithelial Organoid Maintenance Medium which used specifically for long-term culture. Add 200 µL Human Gastric Epithelial Organoid Supplement B(50x) and 40 µL Human Gastric Epithelial Organoid Supplement C(250x) to 9.76 mL Human Gastric Epithelial Organoid Basal Medium. Mix thoroughly.
   NOTE: If not use immediately, store complete medium at 2-8°C for not more than 2 weeks. bioGenous™ Human Gastric Epithelial Organoid Supplement B contains fungicide and antibiotics(50x).

Protocol for Establishment of Human Gastric Epithelial 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 Organoids from Primary Tissue

1. Collect 1-2 cm^2 primary human gastric 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 or if they also contain fat or muscle tissue. If so, remove non-epithelial components as much as possible using surgical scissors or scalpels and forceps under a dissection microscope. If no fat or muscle tissue are present, continue to the next step immediately.
3. Rinse the gastric tissue with Epithelial Organoid Basal Medium(B213151) or DPBS until the supernatant is clear.
4. Before glands isolation, thaw Matrigel 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 DPBS.
7. Wash the samples by pipetting with a 10 mL pipette at least ten times.
   CRITICAL For the subsequent steps, coat the inner surface of every 10 mL pipette with 10% (vol/vol) FBS medium before use to avoid 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 cold DPBS.
9. Repeat Steps 7 and 8 3–5 times until the supernatant is free of debris.
   CRITICAL Thorough washing of the sample is crucial to avoid bacterial contamination.
10. Add 10 mL of cold DPBS supplemented with 2 mM EDTA (E219121) to the tube. Place the tube on a rocking shaker and rock it gently at 4 °C for 40 min.
11. After treatment with EDTA, stand the tube still until the samples settle to the bottom of the tube, and then aspirate the supernatant with a 10 mL pipette and add 10 mL of cold DPBS.
12. Stand the tube still until the samples settle at the bottom. Aspirate the supernatant with a 10 mL pipette.
13. Add 10 mL of cold DPBS and pipette up and down at least ten times with a 10 mL pipette. Allowed the tissue fragments to settle down under normal gravity for at least 30s. The glands will be released into the supernatant by pipetting. Place the supernatant containing the isolated glands into a new 15 mL tube.
14. Spin the glands at 4°C at 300g for 3 min. Remove the supernatant and place the tube on ice.
15. Resuspend the pellet in 1 mL of DPBS and transfer the glands suspension into a new 1.5 mL tube. Drop 20 µL of the gland suspension on a petri dish. Count the number of glands under a stereomicroscope and calculate the total number of glands.
16. Spin the glands at 4°C at 300g for 3 min. Aspirate and discard the supernatant.
17. Aspirate the supernatant and resuspend the pellet in Matrigel. Matrigel should be kept on ice to prevent it from solidifying; thus, work quickly. The amount of Matrigel depends on the size of the pellet. Approximately 100-250 glands should be plated in 25 µL of Matrigel.
    CRITICAL Do not dilute Matrigel too much (Matrigel should be >70% (Matrigel vol/Total vol)) to ensure formation of solid droplets.
18. Plate the Matrigel containing organoids on the bottom of 24-well cell culture plates in droplets of 25 µL each around the center of the well.
    CRITICAL Once the organoids are resuspended in Matrigel, proceed with plating as quickly as possible, as the Matrigel may solidify in the tube or pipette tip. Do not let the Matrigel touch the wall of wells.
19. Place the culture plate into a humidified incubator at 37 °C and 5% (vol/vol) CO_2 for 15-25 min to let the Matrigel solidify.
20. Prepare the required amount of Human Gastric Epithelial Organoid Primary Culture Medium.
21. Once the Matrigel droplets are solidified (15-25 min), open the plate and carefully add 500 µL of Human Gastric Epithelial Organoid Primary Culture Medium to each well.
    CRITICAL Do not add the medium directly on top of the Matrigel droplets, as this might disrupt the droplets.
22. Place the culture plate in a humidified incubator at 37 °C and 5% (vol/vol) CO_2.
23. Change the medium every 3 days by carefully aspirating medium from the wells and replacing it with fresh, pre-warmed medium.
24. Closely monitor the organoid formation. Ideally, human gastric epithelial organoids should be passaged for the first time between 5 and 8 days after initial plating.

Splitting and Passaging of Organoids

1. Pipette up and down to disrupt the Matrigel, and transfer the organoid suspension into a 1.5 mL conical tube.
2. Pipette the organoid suspension up and down to mix thoroughly. Use the bottom of the tube to create pressure, which will aid the removal of Matrigel.
3. Centrifuge organoids at 300g for 3 min at room temperature.
4. Aspirate the supernatant, and split organoids using either mechanical disruption or Organoid Dissociation Solution (E238001). For mechanical disruption, resuspend the pellet in 1 mL of Organoid Basal Medium. Use a pipette tip to pipette the organoid suspension up and down 30 times. Use the bottom of the tube to create pressure, which will aid organoid disruption. In case of 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 ≥10 times every 1 min to aid in the disruption of the organoids. Monitor digestion closely to keep the incubation time in Organoid Dissociation Solution to a minimum.
CRITICAL Do not dissociate in Organoid Dissociation Solution for >3 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.

5. After shearing is complete, wash once by topping up with 1 mL of Organoid Basal Medium, and centrifuge at 300g for 3 min at room temperature.
6. Aspirate the supernatant and resuspend the organoid pellet in 70% (vol/vol) Matrigel, and plate organoids in droplets on the bottom of a culture plate as described in Steps 17. After plating is complete, transfer the plate into a humidified incubator at 37 °C and 5% (vol/vol) CO_2 for 15–25 min.
7. Pre-warm Human Gastric Epithelial Organoid Maintenance Medium at 37 °C.
8. After the Matrigel droplets have solidified (15–25 min), carefully pipette pre-warmed medium into the wells.
9. Place 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 27th June, 2022