

## bioGenous™ Human Esophageal Organoid Kit (Serum-free)

Catalog: K2040-HES

### Product Description:

bioGenous™ Human Esophageal Organoid Kit is a serum-free cell culture medium for Human Esophageal organoids derived from adult stem cells. Self-renewal of the esophageal is driven by the proliferation of stem cells and squamous cells. Human Esophageal organoids display all hallmarks of the esophageal in terms of architecture, cell type composition, and self-renewal dynamics, therefore hold great promise for unprecedented studies of Human Esophageal homeostasis and disease.

### Product Information:

| Component   | Component Cat#      | Volume        | Storage & Stability                                 |
|---|---------------------|---------------|---|
| bioGenous™ Human Esophageal Organoid Basal Medium       | K2040-HES-A100/A500 | 100 mL/500 mL | 4°C, 12 months                                      |
| bioGenous™ Human Esophageal Organoid Supplement B(50x)  | K2040-HES-B100/B500 | 2 mL/10 mL    | -20°C, avoid repeated freeze-thaw cycles, 12 months |
| bioGenous™ Human Esophageal Organoid Supplement C(250x) | K2040-HES-C100/C500 | 0.4 mL/2 mL   | -20°C, 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™ | Organoid Dissociation Solution                      | E238001  |
| bioGenous™ | Anti-Adherence Rinsing Kit                          | E238002  |
| bioGenous™ | Organoid Cryopreservation Medium (Serum Free)       | E238023  |
| bioGenous™ | Organoid Culture ECM (Reduced Growth Factor)        | M315066  |
|            | Fetal Bovine Serum (FBS)                            | -        |
|            | DPBS (1X), liquid, contains no calcium or magnesium | -        |

## Preparation of Human Esophageal Organoid Primary Culture Medium and Maintenance Medium

Use sterile technique to prepare the Human Esophageal Organoid Primary Culture Medium and Maintenance Medium. The following examples are for preparing 10 mL of Primary Culture Medium and Maintenance Medium. If preparing other volumes, adjust accordingly.

1. Thaw Human Esophageal Organoid Supplement B (50x) and Human Esophageal 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 200 µL Human Esophageal Organoid Supplement B (50x), 40 µL Human Esophageal Organoid Supplement C (250x) to 9.76 mL Human Esophageal 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 Esophageal Organoid Supplement B contains fungicides and antibiotics (50x).

### Protocol for Establishment of Human Esophageal 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 primary Human Esophageal 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 is present, continue to the next step immediately.
3. Rinse the tissue with Epithelial Organoid Basal Medium (B213151) until the supernatant is clear.
4. Thaw bioGenous™ Organoid Culture ECM (M315066) on ice and keep it cold.
5. Mince the tissue into small fragments 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.

Digest the tissue fragments with 10mL of Tissue Digestion Solution(K601008) in a 15mL conical tube at 37°C, with a variable incubation period ranging from 50 min to 1 h. Carefully monitor the digestion process by mixing the content of the tube every 5-10 min by shaking vigorously and pipetting the mixture up and down.

**CRITICAL** To prevent over-digestion, one should examine the cells under the microscope if the epithelium cell clusters appear during digestion.

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 3 min and centrifuge at 250g for 3 min at 4°C.
8. Aspirate the supernatant and resuspend the pellet in Epithelial Organoid Basal Medium, centrifuge at 250g for 3 min at 4°C, and repeat this step one more time.
9. 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.
10. 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.
11. Place the culture plate into a humidified incubator at 37 °C and 5% (vol/vol) CO<sub>2</sub> for 15-25 min to let the ECM solidify.
12. Prepare the required amount of bioGenous™ Human Esophageal Organoid Medium.
13. 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.
14. Place the culture plate in a humidified incubator at 37 °C and 5% (vol/vol) CO<sub>2</sub>.
15. Change the medium every 3 days by carefully aspirating the medium from the wells and replacing it with fresh, pre-warmed Human Esophageal Organoid Medium.
16. Closely monitor organoid formation. Ideally, Human Esophageal organoids should be passaged for the first time between 7 and 10 days after initial plating.

### Splitting and Passaging of Organoids

1. Pipette up and down to disrupt the ECM, 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 ECM.
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) ECM, and plate organoids in droplets on the bottom of a culture plate as described in Steps 10. After plating is complete, transfer the plate into a humidified incubator at 37 °C and 5% (vol/vol) CO<sub>2</sub> for 15–25 min.
7. Pre-warm Human Esophageal Organoid Maintenance Medium at 37 °C.
8. After the ECM droplets have solidified (15–25 min), carefully pipette the pre-warmed medium into the wells.
9. Place culture plates in a humidified incubator at 37 °C and 5% (vol/vol) CO<sub>2</sub> until the organoids are needed for further experiments.

Last updated on 23<sup>th</sup> Jan 2024