

# Regenix Uterus protocol



Regenix Uterus is composed of various basement membrane proteins separated from the uterus tissues. Regenix Uterus can be utilized for two-dimensional (2D) and three-dimensional (3D) culture of endometrial cells. In particular, Regenix Uterus can provide an optimized environment for adult stem cells (AdSCs)-derived endometrial organoids.

## Storage Instructions

- ⊙ Avoid storing Regenix Uterus on freezer doors or in frequently opened freezers.
- ⊙ After the initial thaw, aliquot Regenix Uterus into freezer-compatible tubes and store at  $-80^{\circ}\text{C}$ . Minimize repeated freezing and thawing to maintain product quality.
- ⊙ Long-term storage after thawing is not recommended for optimal product integrity.
- ⊙ Frozen Regenix Uterus is stable for up to 2 years from the date of manufacture.

## Thawing Instructions

- ⊙ Regenix Uterus begins to gel at temperatures above  $10^{\circ}\text{C}$ .
- ⊙ Thaw for at least 4 hours at  $2^{\circ}\text{C}$  to  $8^{\circ}\text{C}$ , ensuring the vial is fully surrounded by ice.
- ⊙ During thawing, keep the ice bucket covered and place it in a cold room or at the back of a refrigerator for consistent temperature control.

## Instructions for 3D Culture of Endometrial Organoids

### ⊙ Preparation of Regenix Uterus

Thaw Regenix Uterus and gently mix by slow pipetting. Due to its high viscosity, use a 200  $\mu\text{L}$  pipette tip cut to a 1.5–2 mm opening to reduce bubble formation. If bubbles occur, centrifuge before use. Keep Regenix Uterus at  $4\text{--}8^{\circ}\text{C}$  during handling to prevent gelation above  $10^{\circ}\text{C}$ .

### ⊙ Organoid Resuspension

Before adding Regenix Uterus, carefully remove as much supernatant as possible from the prepared organoid pellet. Then, add Regenix Uterus and gently mix by slow pipetting to ensure uniform resuspension. Regenix Uterus is provided as a ready-to-use pre-gel solution. Dilution is not recommended, as it may prevent proper hydrogel formation.

### ⊙ Gelation

Dispense 30  $\mu\text{L}$  of the mixture into each well of a 48-well plate and incubate at  $37^{\circ}\text{C}$  for 40 minutes to allow gel formation.

## ⊙ Medium Addition

Carefully add the appropriate volume of medium. If adding 300  $\mu$ L per well, dispense the medium slowly over 15 seconds to prevent disruption of the gel. The culture of organoids with Regenix Uterus may require the addition of 10  $\mu$ M Y-27632 during the first 1–2 days.

## Subculture Instructions for Endometrial Organoids

### ⊙ Preparation of Collagenase Solution

Prepare a 2 mg/mL solution of collagenase IV (600–800 U/mL) in basal medium. Other types of collagenase can be used, but their concentrations may need optimization.

### ⊙ Detachment of Regenix Uterus Droplets

Gently touch the side of the Regenix Uterus droplet with a 1000  $\mu$ L pipette tip to detach it from the bottom of the well plate.

### ⊙ Transfer of Regenix Uterus-Encapsulated Organoids

Cut off the tip of a 1000  $\mu$ L pipette tip with sterile scissors to create a 2.5–3 mm opening. Use this modified tip to transfer the Regenix Uterus-encapsulated organoids into a 15 mL conical tube. Using a 15 mL conical tube is recommended to prevent the organoid pellet from sticking to the microtube walls.

### ⊙ Collagenase Treatment

Carefully remove the supernatant and add enough collagenase IV solution to fully submerge the Regenix Uterus droplets (e.g., use 1 mL of collagenase IV solution per 6–8 Regenix Uterus droplets).

Incubate the 15 mL conical tube upright at 37°C for 1 hour. Do not exceed 1 hour, as prolonged incubation may damage the organoids.

### ⊙ Removal of Regenix Uterus and Washing

After 1 hour, a thin layer of Regenix Uterus will remain above the organoid pellet. Carefully aspirate this layer and wash the organoids twice with basal medium.

### ⊙ Re-Encapsulation

Re-encapsulate the organoids in Regenix and continue culturing under the same conditions as before.