# Regenix Heart protocol



Regenix Heart is composed of various basement membrane proteins separated from the Heart tissues. Regenix Heart can be utilized for two-dimensional (2D) and three-dimensional (3D) culture of cardiomyocytes. In particular, Regenix Heart can provide an optimized environment for cardiac spheroids composed of pluripotent stem cells (PSCs)-derived and directly reprogrammed cardiomyocytes.

#### **Storage Instruction**

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

# **Thawing Instruction**

- © Regenix Heart begins to gel at temperatures above 10°C.
- ⊙ Thaw for at least 4 hours at 2°C to 8°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 Cardiac Spheroids

#### O Preparation of Regenix Heart

Thaw Regenix Heart and gently mix by slow pipetting. If bubbles occur, centrifuge before use. Keep Regenix Heart at 4–8°C during handling to prevent gelation above 10°C.

# O Cell Resuspension

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

#### Gelation

Dispense 30-40  $\mu$ L of the mixture into each well of a 48-well plate and incubate at 37°C for 40 minutes to allow gel formation. For suspension cultures, dispense Regenix Heart droplets on a sheet of Parafilm for easy detachment after gelation.

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# 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. For suspension cultures, solidified Regenix Heart droplets are removed by creating a gentle flow of medium over the parafilm to move the droplets into the 24-well plates.