

# Isolation and culture of endothelial cells from mouse aorta

## Preparation of aorta rings

1. Euthanize mice by CO<sub>2</sub> asphyxiation followed by cervical dislocation.
2. Wipe off skin with 70% isopropanol, and fix mice on an operation table.
3. Cut off tissue to open chest and abdomen, and expose aorta.
4. Dissect thoracic aorta out, and put it into PBS washing solution in 35-mm petri dishes.
5. Under a stereomicroscope, remove connective tissue around adventitia.
6. Transfer aorta to new petri dishes, and cut it to 1~2 mm cross-sectional slices (rings) using an iris scissors.

## Implantation of aorta rings in matrix

1. Coat new petri dishes with matrix by adding 50 µl diluted Matrigel solution.
2. Allow Matrigel to polymerize at room temperature for 20 min.
3. Place aorta rings on the Matrigel layer, and cover the aorta tissue with a few drops of Matrigel.
4. Allow Matrigel to polymerize at room temperature for 20 min.
5. Wash once with *pre-warmed* PBS.
6. Add *pre-warmed* culture medium, and allow endothelial cells (EC) to sprout and grow for 5~7 days at 37 °C in a humid incubator chamber with 5% CO<sub>2</sub>.

## Isolation of sprouting EC

1. When a large number of cells sprouting from aorta are observed, remove aorta rings from Matrigel with a sterile fine-tip forceps under a stereomicroscope.
2. Wash remaining Matrigel and cells with PBS twice.
3. Add dispase solution (0.1 ml/cm<sup>2</sup>), and incubate for 20 min at 37 °C in a humid incubator chamber.
4. Pipette solution up and down, and transfer solution into a 15-ml tube.
5. Add *pre-chilled* 10 ml culture medium, and centrifuge at 500 X g for 10 min at 4 °C.
6. Discard supernate, and suspend the cell pellets with 1 ml culture medium.

## EC culture

1. Coat a dish/flask with fibronectin (0.2 ml/cm<sup>2</sup>) at 4 °C overnight, and wash twice with PBS.
2. Add EC suspension and culture medium to the dish/flask.
3. Change medium every 3 days and split cells when reaching ~90% confluence.

## Appendix

**PBS washing buffer** PBS with antibiotics (50 µg/ml penicillin and streptomycin)

**Matrigel solution** Add pre-chilled PBS into Matrigel (BD Biosciences, Cat# 356231) at 1:1 on ice.

**Dispase solution** Dispase I (Sigma Cat# D4818, 2 U/ml) in PBS.

**Fibronectin solution** Fibronectin (Sigma Cat# F1141, 10 µg/ml) in PBS.

**Culture medium** Ham's F12/DMEM medium with 5~10% FBS (5% for long-term culture), 50 µg/ml ECGS (endothelial cell growth supplement, made from bovine hypothalami or Sigma Cat# E2759), 10 U/ml heparin (Sigma Cat# 9399), 50 µg/ml penicillin, 50 µg/ml streptomycin.

