

Vascular smooth muscle cell (VSMC) isolation from mouse aorta

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Abstract

This is a method for VSMC isolation from mouse aorta by an explant method adapted from Metz et al (2012).

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Protocol

Mouse preparation

Step 1.

Sacrifice mouse (10-week old, Balb/c) with an overdose intraperitoneal injection of sodium pentobarbital.

Mouse preparation

Step 2.

Dip the mouse into a beaker filled with 70% ethanol, fix into an operation tray, bring into laminar air flow.

Aorta isolation

Step 3.

With sterilized surgical instruments, open the mouse, dissect the thoracic aorta.

Aorta isolation

Step 4.

Wash the aorta in Hank's balanced salt solution (HBSS).

Aorta isolation

Step 5.

Keep the aorta in a dish on ice, remove the connective tissue and the adventitia.

Aorta isolation

Step 6.

Transfer into a new dish with fresh HBSS.

Aorta isolation

Step 7.

Cut open the aorta longitudinally, remove the endothelial layers by gentle scrape with a sterile cotton swab.

Aorta isolation

Step 8.

Cut the aorta into 4 pieces (around 4 mm² each), wash in Dulbecco's modified Eagle's medium/Ham's F12 (DMEM/F12) (Gibco).

Media explant

Step 9.

Transfer the aorta pieces into a collagen-coated culture dish (35 mm), press with the luminal side down on the dish. Spread the aorta out over as large as possible.

📌 NOTES

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Prepare the collagen-coated culture dish in advance. Add 1-2 ml collagen, incubate 30-60 minutes at room temperature, rinse with PBS three times, keep the last PBS up to use.

Media explant

Step 10.

Incubate for 5 min in a 37°C, 5% CO₂ incubator in a tilting position.

🌡 TEMPERATURE

37 °C Additional info: 5% CO₂ incubator

Media explant

Step 11.

Check and make sure the media explant attached to the dish. Prolonged the incubation for another 1-3 minutes if necessary.

📌 NOTES

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Be careful not to dry the media explant.

Media explant

Step 12.

Very slowly add fresh medium supplemented with 20% fetal bovine serum (FBS) (Sigma) and antibiotics (70 mg/ml penicillin and 50 mg/ml streptomycin) it just covers the aorta. Start with 100 µl

up to 500 µl.

NOTES

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Make sure not to disturb the attachment of explants to the dish.

Media explant

Step 13.

Place the dish in the incubator and left undisturbed for 4 days.

Media explant

Step 14.

At day 5, add fresh medium and then change every 3 days. Observe the cells that had migrated from the medial explant under a microscope when changing medium.

Culture of VSMCs

Step 15.

When the migrated cells from media explants are confluent, harvest with trypsinization.

Culture of VSMCs

Step 16.

Count the cells and pool cells from media explants from 3 mice into one dish ($0.5-1 \times 10^5$ cells/35 mm dish), culture in 10%FBS/DMEM/F12 (complete medium) as needed thereafter for experiments. This is passage 1.