

ISOLATION OF ENDOTHELIAL CELLS FROM MOUSE EMBRYOS

- Pregnant mouse is sacrificed by cervical dislocation 9.5dpc, the uterus is removed and the embryos are isolated.
- After removing deciduas and Reichert's membrane under a stereomicroscope, the embryos are individually transferred in test tubes Falcon 2054 with 1 ml/test tube of a collagenase-DNase solution (respectively 1.5mg/ml and 25µg/ml in Hepes buffered DMEM w/o serum). The incubation is performed in a bain-marie at 37°C for 30min with occasional gentle pipetting.
- The obtained cell suspension is then spun in an eppendorf centrifuge for 10 sec. and the cells are recovered in 1ml of fresh culture medium.
- An aliquot of the cell (150µl) is centrifuged in an eppendorf test tube and washed with 1ml PBS; the supernatant is discarded and the pellet frozen on dry ice for the following genotyping. The remaining aliquot (850µl) is seeded in a gelatin (0.1%)-coated 48-well plate (one embryo/well). The culture medium is DMEM-Hepes buffered supplemented with 20% FBS, Penicillin (100U/ml), Streptomycin (100µg/ml), ECGS (50µg) and Heparin (100µg/ml).

Cell infection

- 48-72h later after seeding, the culture medium is removed and replaced with 500µl/well supernatant of GgP+E prepared as described in the protocol "Polyoma middle T production...", with the addition of 8µg/ml polybrene.
- The cultures are incubated at 37°C. After 4 hours the medium is removed and replaced with 1ml of fresh complete medium.
- (Optional) The following day the infection with the GgP+E supernatant is repeated.
- Cultures are inspected daily.
- After reaching a tight confluent monolayer, the cells are washed with PBS, detached with a trypsin-EDTA solution and subcultured with a split ratio 1:2.
- Endothelial cells are routinely cultured on 0.1 % gelatin -coated plasticware.

Materials

Collagenase A (Roche Diagnostics GmbH, Cat. No. 103578)

DNase (Roche Diagnostics GmbH, Cat. No. 104 159)

DMEM Hepes buffered (Gibco Life Technology LTD, Cat. No. 42430025)

Penicillin-Streptomycin sol. (100x) (Gibco Life Technology LTD, Cat. No.15140-114)

FBS (HyClone Laboratories, Cat. No. SH 30070.03)

ECGS (Sigma Chemical Co, Cat. No. E0760)

Heparin (Sigma Chemical Co, Cat. No. H3149)

Gelatin from bovine skin (Sigma Chemical Co, Cat. No. G9382)