



Ear and Tail fibroblast culture and digestion

Collagenase solution/Digestion medium

DMEM HG 15% FBS + Collagenase (200 u/ml type IV, sigma C5138) + Dnase I (25 Kunitz/ml, sigma D-4263) + antibiotics (1 ug/ml gentamicin, 10 mg/ml stock)

Tissue prep.

1. Wipe tail or ear tissue with 70% ethanol.
2. Wash tail or ear tissue with sterile PBS.
3. Mince tissue in 10 mm plate.
4. Add 20 ml digestion medium.
5. Keep the plate in incubator (5% 39 degrees) for 7-8 hours for tissue digestion.
6. Transfer medium plus tissues to 50 ml tube centrifuge 500 g for 5 minutes.
7. Remove supernatant.
8. Add 10 ml cell culture medium, resuspend, centrifuge again, remove supernatant.
9. Add 40 ml cell culture medium, transfer to two 250 ml tissue culture flasks.
10. Culture for 24 hours, cells should be confluent and ready to freeze for future use.

Freeze primary cells

1. Freezing medium is FBS +10% DMSO.
2. Wash cells with PBS.
3. Trypsin treat cells (0.05%) and transfer to incubator for 3-5 minutes.
4. Stop trypsinization by adding DMEM with FBS.
5. Centrifuge the suspension 5 minutes 1500 rpm, remove supernatant.
6. Resuspend in freezing medium.
7. Add 250-500 ul/1 ml cryovial.

Culturing Fibroblast cells

1. Thaw cells.
2. Centrifuge 5 minutes 1500 rpm in DMEM HG 15% FBS.
3. Remove supernatant, resuspend and plate as needed (depending on cell concentration).