



Fetal fibroblast culture and digestion

Collagenase solution

DMEM HG 15% FBS+ Collagenase (200u/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. Mince tissue in 10 mm plate
2. Add 20ml digestion medium
3. Keep the plate in the incubator (5% 39 degrees) for 7-8 hours for tissue digestion.
4. Transfer medium plus tissues to 50 ml tube centrifuge 500 g for 5 minutes
5. Remove supernatant
6. Add 10 ml cell culture medium resuspend centrifuge again, remove supernatant.
7. Add 40 ml cell culture medium transfer to two 250 ml tissue culture flasks
8. Culture for 24 hours, cells should be confluent and ready to freeze for future use.

Freeze primary cells

1. Freezing medium FBS +10% DMSO
2. Wash cells with PBS
3. Trypsin treat cells (0.05%) and transfer to incubator for 5-10 minutes
4. Stop trypsinization by adding DMEM with FBS
5. Centrifuge the suspension 10 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 10min 1500 rpm in DMEM HG 15% FBS
3. Remove supernatant resuspend and plate as needed (dependent on cell concentration).