



Isolation of Ovine Embryonic Fibroblasts

1. Warm growth media (15ml), trypsin (7ml), PBS+P/S (15ml) and outgrowth media (30ml) (volumes per embryo)
2. Harvest embryo and transfer into 15ml of growth media in a 50ml falcon – keep at room temperature
3. In sterile flow hood decapitate the embryo and eviscerate the body.
4. Wash x3 using a total of 15ml PBS+P/S
 - 4.1. By pipetting 5ml PBS+P/S into 3 sterile petri dishes
 - 4.2. add the embryo and swirl gently
 - 4.3. Continue until 3 washes have been carried out
5. Transfer embryo to a 15ml falcon
6. Add 5ml trypsin and incubate at 37°C for 5 minutes.
7. Vortex for 30 secs to 1 minute
8. Incubate at 37°C for a further 5 minutes
9. Remove 3ml of the trypsin/cell mix (avoiding any large clumps) and pass the mix through a 100uM cell strainer (yellow) into a 50ml Falcon tube.
10. Once the suspension has passed through the strainer, rinse the strainer with 6ml of fibroblast outgrowth medium.
11. Transfer the cell suspension into a 15ml falcon tube
12. Centrifuge for 3 minutes at 200 x g
13. Resuspend cell pellet in 9ml (1ml then +8ml) outgrowth medium
14. Split the 9ml cell suspension between 3 T75 flasks (i.e. 3ml suspension in each flask)
15. To the remaining 2ml of trypsin + embryo, add another 2ml trypsin and continue from Step 6 onwards.
16. Incubate flasks in outgrowth medium for 5-7 days (keeping the cells in the outgrowth medium helps the fibroblasts to outgrow keratinocytes and gives the antibiotics/antifungal time to kill of anything that might be in your embryos). Don't move or disturb the flasks for the first 5 days.

17. When the flasks are 80-90% confluent, passage them 1:2 into standard fibroblast growth medium or freeze down.

OEF Media

Fibroblast outgrowth medium		500ml
DMEM, high glucose, glutamine, pyruvate		382.5ml
FBS	20%	100ml
MEM NEAA	1x	5mls (100x)
Pen-strep	1%	5ml
Fungizone	2.5ug/ml	5ml (250ug/ml)
Gentamicin	50ug/ml	2.5ml (10mg/ml)

Fibroblast growth medium		500ml
DMEM, high glucose, glutamine, pyruvate		440ml
FBS	10%	50ml
MEM NEAA	1x	5ml
Pen-strep	1%	5ml

PBS+P/S		500ml
PBS		498.5ml
P/S	X1	1.5ml