

Murine Fibroblasts (from tail clip)

Limitations: 1) Cells begin to senesce after several (less than 5) divisions

Use 1 tail from adult animal which should be sufficient to expand for 1 or 2 experiments, depending on how well they passage and grow (can vary depending on prep). Use mice that have been housed in the barrier to avoid mycoplasma.

1. Clip 1cm of tail from mouse.
2. Soak 10min in Complete Media + 5x pen/strep.
3. Rinse in Complete media with no pen/strep.
4. Mince tails with EtOH soaked razor blade and transfer to 6 well dish containing 1ml (500 units) collagenase + 1ml Complete Media. If does not cover, can increase volume keeping proportions the same.
5. Incubate overnight at 37°C.
6. Next am, disperse cells by pipeting up and down (5ml pipet).
7. Transfer to 15ml tube and spin 5min at 1500rpm.
8. Take up cell pellet in .5ml media and add to 4.5ml in T25.
9. Leave undisturbed for 48h.
10. Passage cells (by trypsinization) transferring all cells to T75 and grow to confluence to expand cells for experiment. Depending on yield and growth rate sometimes flasks are full in 24 hrs. If so, can expand into 2 T75s.

Complete Media

DMEM (high glucose)

20% FCS

2mM glutamine

1mM non-essential amino acids

1x pen/strep (see above)

Collagenase (Wako 034-10533 in 200U/mg)

500U/ml for fibroblasts prep

2.5mg/ml HBSS or MEM-Joklik (from cardiocyte prep)