

Skin-derived Fibroblast Culture Procedure:

- The skin biopsy is cut into 3-4 smaller pieces (in petri dish with a sterile knife)
- Put 2 ml medium* in sterile T25 flask (with filter top)
- Remove medium from T25 flask (the bottom of the flask has to remain a bit wet)
- Put skin pieces carefully on bottom of flask; spread the pieces
- Add very carefully 0.5 ml medium
- Transfer flask to 37 degrees Celsius, 5% CO₂ -> leave flask for a minimum of 3 days
- After a minimum of 3 days, cells start growing around the skin biopsy; these cells are not yet fibroblasts, but then you know the skin piece is really attached to the bottom of the flask. When you see these cells you can carefully add some extra medium (1 ml medium). If you do not see these cells yet, it is better to add only 0.5 ml medium to prevent the skin pieces from starting to float
- Look at T25 flask every 2-3 days. You may add additional medium until 5 ml medium in total. When no cells are yet growing around the skin pieces, add only 0.5 ml medium every time very carefully. Finally, when you have added 5 ml medium in total, you have to refresh the medium every 2-3 days. Probably, fibroblasts are then growing out of the initial cell layer.
- If necessary, because the fibroblasts start growing only from 3-4 sites in the T25 flask, an internal trypsinization step can be performed to spread the fibroblasts over the whole T25 flask (we add 0.5 ml 2x trypsin to T25 flask -> when the cells are detached, add 4.5 ml medium)
- When T25 flask is completely filled with fibroblasts, transfer fibroblasts to T75 flask (0.5 ml 2x trypsin + 9.5 ml medium)
- When the T75 flask is completely filled, we freeze half of the cells and put the other half of the cells in a new T75 flask (we repeat this step a few times, until we have at least 4 vials from different passages).

* MEDIUM: DMEM-F12 medium with glutamax from Invitrogen (31331). Add 1% pen/strep, 1% HEPES, 1% sodium pyruvate, 20% heat-inactivated fetal calf serum (all from Invitrogen)