

## Primary fibroblast culture from human skin biopsy

### Reagents

#### Transport ion medium:

RPMI 1640	450 ml
FBS	50 ml (final as 10%)
Penicillin-streptomycin Solution 100x	1 ml (final as 0.2%)

#### Human Primary Fibroblast culture medium

RPMI 1640	450 ml
FBS	50 ml (final as 10%)
Penicillin-streptomycin Solution 100x	5 ml (final as 1%)

#### Collagenase Solution (filtered for sterilization):

Collagenase type 2 (Worthington cat#4176)	100 mg
Fibroblast culture medium	12.5ml

### Procedure:

1. Skin biopsy should be delivered to lab in transportation medium. Once the sample is received, primary culture should be done as soon as possible. If primary culture could not be done on the same day, keep the sample at room temperature overnight.
2. In the laminar flow hood, transfer muscle biopsy to a sterile 35mm petri dish.
3. Rinse the skin biopsy in petri dish with sterile 1x PBS to remove blood and debris. Remove the adipose tissue with a scalpel.
4. Add 2 ml collagenase solution and mince the tissue with scalpel.
5. Incubate at 37°C for 1 hour.
6. Transfer all of the digested tissue to a 15 ml conical tube, rinse the petri dish with 2ml medium twice and collect the medium in the same tube.
7. Spin down at 200g for 5 minutes at room temperature.
8. Discard supernatant, and wash the pellet with 3 ml medium twice to remove the collagenase, spin down and remove supernatant after each wash.
9. Re-suspend the pellet with 5 ml medium, and transfer it to T25 flask.
10. Incubate the flask at 37°C with 5% CO<sub>2</sub>.
11. Usually fibroblasts can be seen in 1-3 days after initial set up. Some small tissue pieces may also attached to the substrate and fibroblast will migrate from.
12. When there are sufficient cells, detach the cells with trypsin and plate them in another petri dish for further proliferation. If there are still tissue pieces attached, add fresh medium to the original flask, fibroblasts will migrate out continually.