

## **A. Preparing Primary Cultures from Muscle Biopsy Specimens II**

This is the present protocol, it has been used since 6/05

Aspirate transport media and wash specimen with 10 ml PBS.

Aspirate PBS and replace with 5ml PBS.

Pour specimen into sterile Petri dish and cut into small pieces using two sterile #10 scalpels.

Pipette up as much of the specimen as possible and transfer to a 15ml sterile tube. Wash the plate with an additional 5ml of PBS.

Let the specimen settle to the bottom of the tube. Aspirate off PBS, sparing as much of the specimen as possible.

Pipette 10 ml of 1x PBS onto the specimen. Cap tube and shake well. Let the specimen fall to the bottom of the tube, then aspirate off the PBS

Repeat step 5 twice (for a total of three times).

Aspirate the PBS, add 1.6ml of Dispase II solution, 2ml of Collagenase 4 and 40ul of 0.25M CaCl<sub>2</sub>.

Incubate in 37°C waterbath for 15min.

Triturate (pipette up and down) 10 times, try to avoid producing foam, return to waterbath for an additional 15 mins.

Repeat trituration.

If tissue does not appear disassociated (by eye), repeat incubation and trituration.

Place a 100um nylon cell strainer on top of a 50ml tube, prewet filter with PBS.

Pass sample through cell strainer and rinse strainer with an additional 5ml PBS.

Pellet cells, aspirate supernatant and resuspend in 10ml of Primary Culture Media (see below).

Preplate for 60-90 mins on a standard Petri dish (non TC coated). This will allow contaminating fibroblasts to adhere, but myoblasts will not.

Transfer medium to a T75 flask, discard Petri dish.

On the 3rd or 4th day after initiating the culture, supplement the media with 4ul of bFGF in 1 ml of F10 media, myoblasts may not have adhered at this stage and will not have a normal appearance.

For the first day or so, cells will appear rounded and may not be all adhered. It can take up to a week for cells to adhere and flatten out. If original biopsy sample was small or from a severely affected FSHD patient or other disease controls you may not easily see any cells for a few days.

Supplement fresh bFGF every 3-4 days. Change media for 1st time after one week. If it is necessary to perform first media change earlier than 1 week, pellet old media to recover any non- adherent cells and resuspend in fresh media.

Subsequently, change media approx 2x per week until growth foci are seen. Do not allow these foci to overgrow.

Redistribute cells as foci develop. Aspirate medium and rinse with 10ml of PBS, rock flask gently to cover and aspirate. Add 1ml 0.1% trypsin, rock flask to cover surface and either place in incubator or leave at room temp. Cells will begin to detach within a couple of minutes. Gently tap side of flask and check under microscope that cells are detached. Add 10ml of media and return to incubator in same flask.

Grow until culture is approx. 60-80% confluent. Trypsinize as before, split to 3-4 flasks (10ml media per flask). P1

Grow cultures to 60-80% confluence. Trypsinize using 1ml of 0.1% trypsin per flask. When cells are detached inactivate trypsin with 10ml of F10 media (no conditioned media) per flask. Pool media containing cells and pellet at speed 7 for 5mins (use old centrifuge in cell culture lab).

Aspirate supernatant and resuspend cell pellet carefully in freezing medium. Use 2-3ml per flask depending on degree of confluency, (aim for 8-10 vials per line). This is passage 2. Aliquot into cryogenic vials 1ml/vial and freeze slowly (Nalgene Cryo freezing container, 5100-0001) at least overnight and up to a few days. Transfer to liquid nitrogen for long term storage.

Grow cells in F10/conditioned media until just after 1st passage if growing well, or until step 18 (freeze down) if growing slowly. After thawing, Maintenance media may be used.

Aliquot bFGF and store at -20C, store thawed aliquot at 4C for up to one month. Add bFGF at 4ul/10ml media immediately prior to use.

Do not allow cultures to overgrow as they may start to differentiate.

For human lines, label cryovials with subject code, date of freeze and passage #. Do not label vials with subject name. Vials shipped to other labs must have names removed, this is difficult to do on frozen vials with alcohol resistant ink.

To thaw cells, thaw rapidly by holding in a 37C water bath and wash outside of vial with ethanol. Transfer contents of vial to 10ml Maintenance Media in a T75 flask (do not pellet cells at this point, viability will be reduced). Change media the following day to remove DMSO.

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