Myoblast Isolation Protocol – OctoMACS Method

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DAY 1

Place 7mL of 0.2% Collagenase (diluted in DMEM) into a purple capped C-tube

Place ALL of the hindlimb muscles from a single mouse into the C-tube

Secure the cap and perform three cycles of Program Spleen-2 on the OctoMACS

Loosen the cap and dislodge any tissue that may be stuck in the plastic blade

Leave the cap loosen and place the tube + homogenized tissue at 37degrees for 90min

Pipette the solution with a 10mL pipette until you achieve a homogeneous sln (~15-20 times)

Add 100ul of 2% collagenase and 50ul of dispase – pipette up and down to mix

Place at 37 degrees for an additional 30 minutes

Attach a 10mL syringe to a 20-gauge needle

Run your sample through the needle for 10 cycles (1 cycle equals up and then down)

Wet a 40um filter with ~5mL of myoblast media

Run your sample through the filter into a 50mL conical tube

Wash the filter with ~20mL of myoblast media

Spin @1200 for 10 minutes

Aspirate down to the pellet

Resuspend in 1mL of 1X RBC lysis buffer (eBioscience#00-4333-57)

Incubate at RT for 5 min

Add 9mL of FACS buffer and spin 350 x g for 10 minutes. Aspirate down to pellet. Note: there will be a lot of 'stuff' above your small pellet – this is normal

Resuspend pellet in 10mL Myoblast Media + bFGF and plate into a 10cm collagen coated dish.

Check plate in ~1hr. If the prep was 'good' then you should see tons of small very round and refractile cells. Some of these are fibroblasts and some are muscle stem cells.

DAY 2

Aspirate media

Wash once with fresh media and aspirate

Replace with fresh media + bFGF

At this point avoid the temptation to manipulate the cells again until Day 4. Fibroblasts will have begun to 'spread out ' and reveal themselves. Make a note about the ratio of fibroblasts to myogenic cells.

DAY 4

Now you should start to see tons of very round 'myoblasts' in your dish. Note that they are double the size of the tiny 'muscle stem cells' that were in the dish on Days 1 & 2. These cells will continue to proliferate extensively for another few days and we want to capture give them the best opportunity to do so while getting rid of the contaminating fibroblasts. We use the 'pre-plate' method to do this.

Wash dish quickly with 5 mL warm PBS and aspirate.

Add 5ml of warm PBS and swirl to ensure all surfaces are coated.

Place at 37 degree C

In 5 min take the dish out and hit the four 'edges' of the dish to dislodge the loosely adherent myogenic cells. A few of the highly adherent fibroblasts will come along for the ride.

Add 5mL of myoblasts media to 5mL of PBS and spin down all 10mL at 1200rpm.

Aspirate down to pellet and resuspend in 20mL myoblast media + bFGF.

Split evenly into 2 X 10cm collagen coated dishes. Inspect in ~1hr. This is p1.

Day 6

If upon inspection you have tons of myogenic cells and very few fibroblasts, then these cells are ready for use as a myogenic control, etc.

If still quite a few fibroblasts, perform another round of pre-plate. Optimally use p2-p4 cells for a early myogenic control.

Of note, after ~p4, these cells will soon begin to undergo a massive 'crisis' and die. A small proportion of the cells will survive the 'crisis' and will continue to proliferate allowing you to generate a cell line that can be passaged for up to 20 passages. You will need to keep a watchful eye on these cells daily and split often to be sure they are neither too confluent or too subconfluent - both of these conditions will lead to irreversible differentiation of the cells (i.e. exit from cell cycle, fusion with other committed cells)

This population is obviously not representative of the heterogeneity of myogenic cells that you acquire immediately after isolation, but are highly fusogenic and are a good control for many experiments.