DIFFERENTIATION OF 3T3-L1: MDI TREATMENT

N.B: This protocol is performed 2 days after the cells have reached visual confluence.

Reagents:

A. **Insulin**: [10 mg/ml] Sigma cat # I9278-5ML. Stored in refrigerator 5S-11.

B. **Dexamethasone**: (DEX) Solution also probably in fridge (5S-11). If not, powder is in fridge desicator 5S-19. Make in absolute ethanol [stock 0.4mg/ml]. Don't filter! Store at 4°C ~indefinitely.

C. **Methylisobutylxanthine**: (MIX) [11.5mg/ml]. Always made fresh. Add MIX (in desiccator in freezer 5S-19) to 5ml snap cap containing 2ml H_2O. Add 1M NaOH drop wise and vortex until MIX dissolves. Q.S. solution to 3ml and sterile filter. Discard any remaining MIX solution.

Protocol:

1. Use DMEM media with 10% fetal calf serum (fridge 5S-11)
2. Add MIX, DEX, and Insulin:
   MIX = 1ml per 100ml medium
   DEX = 100ul per 100ml medium
   Insulin = 100ul per 100 ml medium
3. Mix well.
4. Remove media from cells and replace with MDI media to initiate differentiation. The day the cells are treated with MDI is referred to as day zero (D0, 0T, or PA)
5. The cells are left in the MDI mix for 48hrs

48 hrs Later (day 2): Remove media and replace with 10% fetal calf serum/DMEM supplemented with full insulin dose (100ul per 100ml medium)

Next feeding (96H, day 4): Feed with 10%FCS supplemented with ¼ insulin dose (25µl per 100ml medium)

Next feeding (164H, day 6) Feed with 10%FCS, No insulin (all feedings after this time are just 10% FCS)

N.B: Cells are typically used for experimentation on day 10.