3T3-L1 Differentiation Protocol

Materials

- Dulbecco’s Modified Eagles Medium (DMEM, GibcoBRL-Cat# 11965-084: high glucose, with L-glutamine, with pyridoxine HCl, without sodium pyruvate)
- Calf Serum (GibcoBRL-Cat# 10437-028/Lot # 1026566)- filter sterilize (0.22µL filter) before mixing into DMEM
- Isobutylmethylxanthine (IBMX; Sigma I-7018)
- Dexamethasone (Sigma D-4902)
- Insulin (Bovine; Sigma I-5500)
- MEM Sodium Pyruvate (100mM; GibcoBRL-Cat# 11360-070)
- Pen/Strep/Glutamine (100x P/S/G; GibcoBRL-Cat# 10378-016

Solutions

- 10% Calf Serum/DMEM
  - 60 mL Calf Serum
  - 6 mL MEM Sodium Pyruvate
  - 6 mL 100x P/S/G
  - 500 mL DMEM

- 10% FBS/DMEM
  - 60 mL Fetal Bovine Serum
  - 6 mL 100 mM MEM Sodium Pyruvate
  - 6 mL 100x P/S/G
  - 500 mL DMEM

- IBMX Solution
  - Dissolve IBMX in a solution made if 0.5N KOH to a final concentration of 0.0115g/mL
  - Filter Sterilize with a .22 mm syringe filter

- MDI Induction Media
  - To required volume
  - 1:100 IBMX
  - 1:100 Insulin
  - 1:1000 Dexamethasone
Method

Preadipocyte maintenance and passage:

Plate the cells in 10% CS/DMEM on treated polysteren culture dishes from Corning and incubate them at 37 °C in 10% CO2. It is important to feed the preadipocytes every couple of days to avoid letting them get to confluent (>70%), if you want to continue to passage them and differentiate them at a later date. Take care to split them appropriately. They can be split as far as 1:15, though it is usually done 1:10 or less depending on need.

Adipocyte Differentiation Protocol

1) Grow preadipocytes to confluency on 10% calf serum/DMEM

2) Two days post confluency (Day 0) stimulate the cells with MDI induction media. You will notice a distinct change in the morphology of the cells (become more spindly) in the next two days

3) After MDI (Day 2) change the media to insulin media. The media will begin to get more viscous as free fatty acids are produced by the cells and secreted into the media.

4) Two days later (Day 4) change the media to 10% FBS/DMEM. Feed cells with 10% FBS/DMEM every two days. Full differentiation is usually achieved by day 8.