

3T3-L1 Differentiation Protocol

by Robin Erickson

MATERIALS

- Dulbecco's Modified Eagles Medium (DMEM; GibcoBRL-Cat# 11965-084: high glucose, with L-glutamine, with pyroxidine HCl, without sodium pyruvate)
- Calf Serum (GibcoBRL-Cat#16170-078/Lot #1060198)
- Fetal Bovine Serum (GibcoBRL-Cat# 10437-028/Lot # 1026566)-filter sterilize (0.22um filter) before mixed 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

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

10% FBS/DMEM

60mL Fetal Bovine Serum (Filter Sterilized)
6mL 100mM MEM Sodium Pyruvate
6mL 100x P/S/G
500mL DMEM

IBMX Solution (make fresh)

- a) Dissolve IBMX in a solution made of 0.5N KOH to a final concentration of 0.0115g/mL.
- b) Filter sterilize through a 0.22 mm syringe filter.

Insulin Stock Solution

167 uM (1mg/mL) in 0.02M HCl
Filter sterilized through 0.22 mm filter
Can store at -20C for long term, 4C short term.

Dexamethasone Stock Solutions

Freezer Stock: 10mM of Dex in 100% ethanol (store at -20C)

Working Stock: Dilute Freezer stock to 1mM in PBS

Filter sterilize and store at 4C.

MDI Induction Media (10mL/10cm plate; 5mL/6cm plate)

To required volume of 10% FBS/DMEM add:

1:100 IBMX

1:1000 Insulin

1:1000 Dexamethasone working stock

Insulin Media (10mL/10cm plate; 5mL/6cm plate)

To required volume of 10% FBS/DMEM add:

1:1000 Insulin

METHOD

Preadipocyte maintenance and passage:

We plate the cells in 10% CS/DMEM on treated polystyrene culture dishes from Corning (Cat#430167) and incubate them at 37C in 10% CO₂. It is important to feed the preadipocytes every couple of days and avoid letting them get too confluent (>70%), if you want to continue to passage them and differentiate them at a later date. So, take care to split them appropriately. They can be split as far as 1:15, though we usually do 1:10 or less depending on need.

Adipocyte Differentiation Protocol:

1. Grow preadipocytes to confluency in 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 2 days.
3. Two days 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 media to 10% FBS/DMEM. Feed cells with 10% FBS/DMEM every two days. Full differentiation is usually achieved by DAY 8.