Growth and Differentiation of 3T3-L1 and 3T3-F442A Cells

(From Ron Kahn Lab at Joslin Diabetes Center)

Materials:

-DMEM-High glucose
-PBS (Ca-Mg free)
-Calf Serum (Hyclone #2151)
-Fetal bovine serum (Hyclone #1115 or Gibco 10437-028)
-Trypsin-EDTA; 0.25% trypsin/1mM EDTA
-DMSO
-Dexamethasone (Sigma #D-1756) 4 mg/ml in ETOH
-MIX; 3-isobutyl-1-methylxanthine (Sigma #I-7018) 5mM stock in PBS, see below about solubilizing in water.
-Insulin

Protocol:

Cells are grown and maintained as fibroblasts in DMEM/High containing **10% Calf serum** in a 10% CO_2, humidified environment at 37 C. Maintain the cells at subconfluency so as not to prematurely arrest cell growth and induce differentiation. It is better to split cells 1:5 or 1:3 every four or five days instead of seeding at low density and feeding.

Differentiation:

For experiments, split at 3.3 x 10^3 cells/cm^2 and maintain until they are completely confluent. If you are unsure, feed them with DMEM/High 10% Calf serum and wait another day. Induce differentiation (Day 0) of confluent cultures by adding differentiation media, MIX-Diff, (see below) for two days. On Day 2 add DMEM/High containing 10% FBS/5 ug/ml insulin. On Day 4 add DMEM/High containing 10% FBS. The cells should be fully differentiated by day 8-10. Cells may be maintained by feeding with DMEM/High containing 10%FBS every 3-4 days. I usually do experiments between day 8-15.

When cells are not differentiating within this time frame they may be too old and fresh, low passage cells should be started.

MIX-Diff media:

-DMEM/High glucose
-10% Fetal bovine serum
-5 ug/ml insulin
-0.4 ug/ml dexamethasone (stock 4 mg/ml in ETOH and stored -20C)
-0.5 mM MIX (Add solid MIX in PBS to give final 5 mM and heat to almost boiling to dissolve, dispense and store at -20C)

Combine above reagents and filter sterilize. Use within 4 weeks.

References:

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