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Here is the "regular" protocol we use for differentiating brown preadipocytes

Typical Differentiation Time Course:

Day 1- split into **Differentiation media**
2
3
Day 4 - induce confluent cells (**Induction media**)
5
Day 6- put back into Differentiation media
7
8- aspirate off old media and add fresh Differentiation media
9
Day 10- fully differentiated cells ready to be used for: Oil Red O staining, RNA/DNA extraction, Western analysis etc.

DIFFERENTIATION MEDIA—

DMEM high
10% FBS
Insulin (final concentration = 20 nano M) (stock = 10 mg/ml = 1.7 milli M, add 1.2 micro liter stock in 100 ml medium = 83,333x dilution)
T3 (final concentration = 1 nano M) (stock = 10 micro M, 10,000x)

INDUCTION MEDIA—

DMEM high
10% FBS
Insulin (final concentration = 20 nano M)
T3 (final concentration = 1 nano M)
Indomethacin (final concentration = 0.125 milli M) (stock = 0.125 M, 1000X)
Dexamethasone (final concentration = 5 micro M) (stock = 2 mg/ml = 5 mM, 1000X)
IBMX (final concentration = 0.5 milli M) (stock = 0.25 M, 500X)

[note: in Klein et al, JBC:274, pp34795-34802, 1999, the first paper describing this protocol, the Dex concentration is 0.5 micro M]