Cell Growth Protocol and Differentiation treatment for the 10T1/2 Cell Line
From: HudsonAlpha/Caltech ENCODE group
Date: September 30, 2011
Prepared by: Brian Williams

C3H 10T1/2 clone 8. Cell culture and differentiation treatment.

The C3H 10T1/2 cell line was originally generated from primary cultures of 14-17 day whole mouse embryos (inbred C3H Heston strain) disaggregated with 0.25% trypsin (Reznikoff et al. (1973) Cancer Research, 33: 3231-3238; Aaronson and Todaro J. Cell Physiol., (1968), 72,2: 141-148. The resulting cell line was subcloned and selected for immortalization and contact-inhibited growth.

Cell culture protocol for cycling (exponentially growing) cells:
Cells are grown at 37°C in a humidified incubator with 5% CO_2.

Fibroblast growth medium:

	final	stock	example
DMEM		100%	420 mL
FBS (fetal bovine serum)	15%	100%	75 mL
Penicillin/Streptomycin	1X	100X	5 mL
Final			500 mL

Materials:

DMEM (high glucose + glutamine + bicarbonate)	GIBCO #11965-084
FBS (fetal bovine serum)	Hyclone #SH30071.03
Penicillin/Streptomycin (100X stock)	Gibco # 15140.

Liquid Nitrogen Storage:
Freeze cells in growth medium supplemented with 10% (v/v) DMSO in 1 ml aliquots of approximately 0.5-1 x 10^6 cells.

Cell culture and passage

Thaw a 1 ml aliquot of cells as quickly as possible in a water bath at 37°C. Transfer cells to 10 mL warm media in a 15 mL conical tube. Spin down at 115 x g at 4°C for 5 minutes. Remove the medium from the tube by aspiration, being careful not to aspirate the cell pellet. Add 10 mL of fresh growth medium, resuspend the cells, and plate on a new 10 cm dish.

2. When cells are 50-60% confluent, split 1:3 (at most). To passage, remove and discard culture medium. Rinse twice with 5 mL PBS (calcium and magnesium free), being sure to drain the dish thoroughly. This allows the trypsin to work more effectively. For a 10 cm dish, add 1 mL of 0.05% (w/v) trypsin + 0.53 mM EDTA solution (Gibco #25300) pre-warmed to 37°C, for 2 minutes. Do not aspirate the trypsin from the dish. Trypsinization is enhanced by swirling the dishes regularly during the interval to keep the trypsin solution dispersed over the cells. Add 5

mL of growth medium to the dish, and dislodge the cells by vigorously pipetting. Collect the cells into a 15 mL centrifuge tube, then check the plate under the microscope to ensure that the cells have been removed. Spin down at 115 x g at 4°C for 5 minutes. Remove the medium/trypsin from the tube by aspiration, being careful not to aspirate the cell pellet. Add fresh medium, resuspend the cells, and then dilute in a larger flask to an appropriate volume (25 mL per 15 cm plate, 10 mL per 10 cm plate) with growth medium and plate on new dishes. It is important when resuspending the cells after spin down that you triturate vigorously. Seeding density becomes non-uniform if the cells are not well-dispersed. Feed again 2 days after plating.

The Nunc plates that we use have the following measurements:

nominal diameter (cm)	actual diameter (cm)	radius (cm)	area (cm^2)	cell plating volume (mL)
15.0	13.5	6.8	143.1	25.0
10.0	8.5	4.3	56.7	9.9
6.0	5.0	2.5	19.6	3.4
3.5	3.4	1.7	9.1	1.6

Be sure to account for these measurements when plating 6 cm immunocytochemistry plates, etc.

2% Equine serum (mock differentiation) treatment

Expose cells to differentiation medium by rinsing fully confluent cells once with PBS and adding 10mL of differentiation medium (for a 10cm dish). Feed with fresh differentiation medium every other day.

Differentiation medium:

	final	stock	example
DMEM		100%	485 mL
Horse serum	2%	100%	10 mL
Insulin	1 µM	1mM	500 µL
Penicillin/Streptomycin	1X	100X	5 mL
Final			500 mL

Materials:

DMEM (high glucose + glutamine + bicarbonate)	GIBCO #11965-084
Horse serum	Hyclone #SH30074.02
insulin	Sigma-Aldrich #I-6634
Penicillin/Streptomycin	Gibco # 15140