Cell Growth Protocol for T-47D Cell Line

From: HudsonAlpha/Caltech ENCODE group

Date: 5/28/2010

Prepared By: Jason Gertz

Growth and Harvest Modifications Addendum to:

"Cell Growth Protocol for T-47D Cell Line" from HudsonAlpha/Caltech ENCODE Group

From: UW ENCODE Group

Date Modified: 11/14/2011

Modified by: T. Canfield and R.S. Hansen (UW)

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T-47D (ATCC #: HTB-133) cell culture and cross- linking

T-47D is a human epithelial cell line derived from an mammary ductal carcinoma. The cells are adherent in culture.

Cell culture protocol

Normal growth medium: RPMI-1640 (Hyclone) + 10% fetal bovine serum (Hyclone) + 100 units/ml penicillin + 100 μg/ml streptomycin

Hormone stripped medium: RPMI-1640 phenol red free (Hyclone) + 10% fetal bovine serum charcoal/dextran treated (Hyclone) + 100 units/ml penicillin + 100 μg/ml streptomycin

Liquid Nitrogen Storage: Normal growth medium supplemented with 5% (v/v) DMSO in 1 ml aliquots of approximately 5 x 10^6 cells.

1. Thaw a 1-ml aliquot of cells as quickly as possible in water bath at 37°C. Transfer cells to 9 ml warm media in 15-ml conical tube. Mix gently. Spin at 1,200 rpm for 5 minutes to pellet cells. Discard media and resuspend pellet gently in 10 ml warm medium. Divide cells into two T-25 flasks containing 5 ml warm media. Place in incubator. After one day, remove the medium and add fresh media.

2. When cells are 70-90% confluent, split 1:3. To do so, remove and discard culture medium. Add 0.25% (w/v) Trypsin + 0.53 mM EDTA (Gibco/Invitrogen) solution at 37°C to barely coat cells and observe cells under an inverted microscope until cell layer is dispersed (usually within 5-15 minutes). Add 2x normal growth medium and collect cells by gently pipetting. Add appropriate aliquots of the cell suspension to new culture vessels.

3. Between four and six days prior to induction, passage the cells into hormone stripped medium in dishes. Change the hormone stripped medium every two days as well as one day before induction.

Cell cross-linking and harvest

4. Trypsinize and count one or two 150mm plates. Plates harvested at 70-90% confluence should contain 1-2 x 10^7 cells.

5. To induce ER, add estradiol to 10 nM, genistein to100nM, bisphenol A to 100nM or DMSO vehicle control to each plate and return cells to incubator. To induce GR, add

dexamethasone to 100nM or ethanol vehicle control.

6. After 1 hour, add formaldehyde to 1% directly to the cells on plates. Swirl to mix. After 10 minutes at room temperature, add glycine to 0.125 M, swirl to mix and leave at room temperature for 5 minutes. Pour off medium and wash with cold PBS, pH 7.4.

7. Add 5 ml cold Farnham Lysis buffer (5 mM PIPES pH 8.0 / 85 mM KCl / 0.5% NP-40) + Roche Protease Inhibitor Cocktail Tablet (Complete 11836145001; for 50 ml, add protease inhibitor tablet just before use) and scrape cells into 15-ml conical tubes. Spin at 1,000 rpm for 5 minutes. Remove supernatant and freeze pellets on dry ice. Store at -80°C.

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SOP: **Propagation of T-47D, Human Breast Ductal Carcinoma Cells**
Date modified: **11/14/2011**
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Growth Medium Modification for T-47D

RPMI-1640 Medium (ATCC, Cat# 30-2001)
10% Characterized FBS (HyClone, Cat# SH30071)
0.2Units/mL Bovine Insulin (insulin from bovine pancreas, cell-culture tested from Sigma-Aldrich, Cat# I6634; 270X stock solution prepared in acidified water per manufacturer's specifications)
Pen-Strep (1X) (Cellgro, Cat# 30-001-CI)

Procedure Modifications

A. Sub-culture

1) Propagate cells until density reaches 70-80% confluence.
2) Aspirate medium.
3) Wash cells with warm 1X PBS.
4) Add 15mL of Accutase and return to incubator for 10-15 minutes, or until cells detach.
5) Immediately remove cells, rinse flask with warm 1X PBS to collect residual cells, and pellet at 500 x g for 5 minutes (4^{o}C).
6) Gently re-suspend cell pellet in warm medium.
7) Perform 1:3 to 1:5 cell split as needed.
8) Record each subculture event as a passage.

B. Maintenance and Generation of Seed Stocks

1) Change media the day after seeding and every 2-3 days thereafter. Use 50mL of growth medium per T225 flask.
2) Following first or second passage after receipt of cells and with sufficient number of cells to continue maintenance and expansion, the major portion of the flasks should be sub-cultured using Accutase as above under "Sub-culture" and a small portion should be set aside as a seed stock. The cell pellet for the seed stock should be resuspended in freezing medium.
3) Cells in freezing medium (growth medium + 10% DMSO) are dispensed into cryovials (2 million cells per 1 mL aliquot) and frozen at -80^{o}C in a Nalgene Cryo 1^{o}C freezing container overnight.
4) Cryovials are transferred the next day to liquid nitrogen freezer for long-term storage.

D. Harvest

1) Passage cells until the desired number of cells is reached.
2) Remove cells from flasks according to protocol described above under "Sub-culture".
3) Examine viability using Trypan blue staining (SOP TP-7).