Cell Growth Protocol for ECC-1 Cell Line

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ECC-1 (ATCC #: CRL-2923) cell culture and cross- linking

ECC-1 is a human epithelial cell line derived from an endometrium adenocarcinoma. 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. When the cells are 70-90% confluent perform a second passage into 150mm plates in hormone stripped medium. Change hormone stripped medium 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 2-3 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.