MCF10A-Er-Src

MCF-10A parental cells ATCC# CRL-10317
MCF10A-Er-Src Debnath et al., 2003 and Iliopoulos et al., 2009

Description of cells

MCF-10A cells are commonly recognized as a ''normal'' breast epithelial cell line, the karyotype is stable and near-diploid, these cells are nonetheless cytogenetically abnormal. These cells harbor a deletion of the locus containing p16 and p14ARF, as well as amplification of MYC. Notably, MCF-10A cells express wild-type p53. MCF-10A cells are negative for estrogen receptor (ER). For details see Debnath et al., 2003.
The MCF10A-Er-Src cells are derived from the MCF-10A parental cells and contain ER-Src, a derivative of the Src kinase oncoprotein (v-Src) that is fused to the ligand-binding domain of the estrogen receptor (ER). For further details see Aziz et al., 1999. Treatment of such cells with tamoxifen (TAM) rapidly induces Src, and morphological transformation is observed within 24-36 hours (Iliopoulos et al., 2009).

MCF-10A ER-Src cells are grown on standard tissue culture plastic in a 5% CO_2 humidified incubator at 37°C. When confluent, MCF-10A ER-Src cells adopt cobblestone morphology, which is typical of mammary epithelial cells. Any deviation from this morphology should be cause for concern; if the cells appear fibroblastic or spindle cell-like, it is unlikely that they will properly form acinar structures in 3D culture. When transformed this cobblestoned morphology changes to be more disrupted and cells start to grow on top of each other. This is only visible after 24-36hrs.

Components for Medium recipes for MCF-10A ER-Src cells
DMEM/F12 (Invitrogen No. 11039-021)
Horse serum (Invitrogen No. 16050-122)
Pen/Strep (100X solution, Invitrogen No. 15070-063)
EGF (Peprotech, 1 mg): Resuspend at 100 ug/ml in sterile ddH2O. Store aliquots at -20°C.
Hydrocortisone: (Sigma No. H-0888, 1-g bottles) Resuspend at 1 mg/ml in 200-proof ethanol and store aliquots at -20°C.
Cholera toxin: (Sigma No. C-8052, 2-mg vials) Resuspend at 1 mg/ml in sterile ddH2O and allow to reconstitute for about 10 min. Store aliquots at 4°C.
Insulin (Sigma No. I-1882, 100-mg vials) Resuspend at 10 mg/ml in sterile ddH2O containing 1% glacial acetic acid. Shake solution and allow 10–15 min to reconstitute. Store aliquots at -20°C.

1L Growth Media
2x 500ml DMEM/F12, 50ml charcoal stripped horse serum (CSHS, 5% final), 10ml pen/strep (1X), 200ul EGF (20ng/ml final), 500ul hydrocortisone (0.5ug/ml final), 100ul Cholera toxin (100ng/ml final) and 1ml Insulin (10ug/ml final)

Resuspension Media
400ml DMEM/F12, 100ml CSHS (final 20%) and 5ml pen/strep

Freeze Media
Growth media plus 10% DMSO and 20% CSHS

For each medium type, premix all of the appropriate additives, sterile filter through a 0.2-lm filer, and add to DMEM/F12 medium bottle.

Preparation of Charcoal stripped horse serum (CSHS)

The following protocol for charcoal stripping fetal bovine serum is taken from "STEROID HORMONES: A Practical Approach" edited by B. Green and R.E. Leake, 1987, IRL Press, Oxford (pages213-214).

Preparation of charcoal-stripped and heat-inactivated fetal calf serum
This procedure will reduce the level of estrogen in the neat FCS to below 10^{-11} M, that is to below 10^{-12} M in the medium containing 10% FCS. Although this level is sufficiently low to permit most studies on estrogen-induced responses, it should not be assumed that such procedures entirely eliminate either the estrogen content or, indeed, the content of any other steroid family.

1. Incubate overnight at, 4C Norit A charcoal [Sigma Prod. No. C3345] and dextran T-70 [Sigma Prod. No. D1390] in 0.25 M sucrose/1.5 mM MgCl2/10 mM Hepes pH 7.4 at final concentrations of 0.25% and 0.0025%, respectively.

2. Take a volume of the dextran-coated charcoal (DCC) equivalent to that of the serum which is to be stripped. Centrifuge it (500 g for 10 min) to pellet the charcoal.

3. Decant the supernatant and replace it with the same volume of FCS. Remember that each new batch of FCS, even from the same supplier, may have different growth characteristics from the last one and must be checked against some of your existing stock.

4. Vortex the tube to thoroughly mix the charcoal with the serum and incubate either for 12 h at 4°C

This is done twice and then filtered.

Thawing conditions
Thaw 1ml vial in 37 water bath, hold vial and shake gently until thawed do not leave in water bath as DMSO will kill cells. Place 1ml of thawed cells into 9ml Harvest media to dilute DMSO and spin at 300g for 5min.
Remove supernantant and resuspend in 1ml of growth media pipet up and down using a 1ml pipet as these cells are very clumpy and need to be resuspended well. Seed out initially into a T25 flask and split when 70% confluent

Subculture

Passaging conditons for a T25 flask/10cm dish

1. Aspirate the Growth Medium, and rinse with 10.0 ml of phosphate-buffered saline (PBS).

2. Aspirate the PBS and add 2.0 ml of 1X (0.5%) trypsin solution (Trypsin 2.5% (10x) 100 ml invitrogen #15090-046 in 500ml PBS). It is not advisable to use high-concentration trypsin solutions when passaging cells also use trypsin without phenol red.
3. Incubate in a 5% CO2 humidified incubator at 37 °C for 15–25 min. Check the extent of trypsinization after 10 min, gently tapping the plate to dislodge the cells. Continue every few minutes. The cells should be completely dissociated from the plate to avoid clonal selection of adherent cells.
5. Once cells are dislodged, add 1.0–2.0ml of Resuspension Medium and pipet to break up cell clumps.
6. Transfer the cells to a 15-ml conical tube and rinse the plate with another 1.0 ml of Resuspension Medium. Add an additional 1.0 ml of Resuspension Medium to the conical tube. Ultimately the cells are resuspended in 3–4 ml. If you are dealing with multiple plates, it is very important to process only one or two plates at a time. The cells will reattach if they are not resuspended in a timely manner after the serum is added.
7. Spin down the cells at 150g in a tissue culture centrifuge for 3–5 min.
8. Aspirate the medium and resuspend the cells in 1.0 ml of MCF-10A Growth Medium. Add 4.0–5.0 ml to the tube, mix the cells, and plate 1.0 ml cells per 10-cm dish in a total of 10 ml of MCF-10A GrowthMedium (1:5 to 1:6 dilution). A 1:5 passage ratio becomes confluent in 2.5 to 3 days, and a 1:6 passage ratio, in 3.5 to 4 days.
9. Keep track of the passage number; cells may start behaving aberrantly in 3D morphogenesis assays starting at passage 35.

Transformation conditions

Cells should be treated with 1uM tamoxifen when 80% confluent. Freshly made tamoxifen (shelf life once reconstituted 1month max at -20°C) should be added to each dish with fresh media. Harvest when necessary complete transformation will plateau at 36hrs.

Tamoxifen sigma # H7904 (4-hydroxytamoxifen).

References:

Aziz, N., Cherwinski, H. and McMahon, M. (1999) Complementation of defective colony-stimulating factor 1 receptor signaling and mitogenesis by Raf and v-Src. *Mol Cell Biol*, **19**, 1101-1115.

Debnath, J., Muthuswamy, S.K. and Brugge, J.S. (2003) Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures. *Methods*, **30**, 256-268.

Iliopoulos, D., Hirsch, H.A. and Struhl, K. (2009) An Epigenetic Switch Involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 Links Inflammation to Cell Transformation. *Cell*.