# Cultivating MCF 10A cells

MCF 10A (ATCC No. CRL-10317) is a non-tumorigenic epithelial cell line with a normal karyotype, derived in 1984 from the mammary gland of a 36-year-old woman with fibrocystic breast disease.
The line is positive for epithelial sialomucins, cytokeratins, and milk fat globule membrane antigen, and is responsive to calcium concentration, insulin, glucocorticoids, cholera enterotoxin, and epidermal growth factor (EGF). Its phenotype varies with culture context: In monolayers, MCF 10A expresses luminal, basal, and progenitor-like markers; in suspension or 3D culture, it forms mammospheres or acinar structures positive for β-casein and α-lactalbumin, displaying luminal and basal characteristics (Qu et al., 2015).

Reviewed: Mar 14, 2023

*This is a bench card. Full protocol available online.*

## *Procedures*

### A › ***Splitting adherent MCF 10A cells***

- ☐ MCF 10A resuspension medium
- ☐ MCF 10A assay medium
- ☐ R0155 20 mg/L Epidermal growth factor, 100 µL

(1.) Prepare the growth medium for the week, but not more. Growth medium is MCF 10A assay medium supplemented with 20 µg/L EGF (1 000 × stock). Follow a rotating schedule of passage and feeding:

| | **Week 1, Week 4** | | | | | **Week 2** | | | | | **Week 3** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Day** | **1, 22** | **3, 24** | **4, 25** | **6, 27** | **7, 28** | **9** | **10** | **12** | **13** | **15** | **16** | **18** | **19** | **21** |
| Schedule A | Tue | Thu | Fri | **Sun** | Mon | Wed | Thu | **Sat** | **Sun** | Tue | Wed | Fri | **Sat** | Mon |
| Schedule B | Thu | **Sat** | **Sun** | Tue | Wed | Fri | **Sat** | Mon | Tue | Thu | Fri | **Sun** | Mon | Wed |
| Action | **Seed** | Feed | Split | Feed | Split | Feed | Split | Feed | Split | Feed | Split | Feed | Split | Feed |
| Passage | P0, 7 | | P1, 8 | | P2, 9 | | P3 | | P4 | | P5 | | P6 | |

> ***Critical:*** Keep the reconstituted growth medium and the EGF stock at 4 °C. Do not refreeze EGF once thawed. Freshly thawed aliquots are stable for up to one month at 4 °C.

(2.) Bring the growth and resuspension medium, PBS, and trypsin to room temperature.

(3.) Aspirate the old growth medium and wash cells once with PBS.

(4.) Cover the cells with 0.25% trypsin/EDTA. Incubate at 37 °C until at least 90% of the cells have detached. 15–20 min

> ***Critical:*** MCF 10A cells adhere strongly. Incomplete detachment may bias the population.

(5.) Add an equal volume of resuspension medium or 1 × trypsin inhibitor. Pipette up and down to dissociate. Squirt the dish surface to detach remaining cells.

(6.) *Critical:* Transfer the cell suspension to a conical tube. Centrifuge at 500 × *g* for 5 min. Aspirate supernatant and resuspend in fresh resuspension medium. Do not skip this step! Count and assess viability.

(7.) Seed the cells at the desired density in a new culture dish. Spread by rocking the dish back and forth.

> ***Critical:*** Do not swirl in a circular motion—this causes the cells to clump in the center of the dish.

### F › *Freezing MCF 10A cells for long-term storage*

- ☐ Cryovial, polypropylene, non-pyrogenic, with seal rings
- ☐ Dimethyl sulfoxide

(1.) Prepare cryo-preservation medium by supplementing resuspension medium (DMEM/F-12 + 20% horse serum) with 8% DMSO.

(2.) Bring the cryo-preservation medium, PBS, and trypsin to room temperature.

(3.) Detach cells from two confluent 10 cm dishes using trypsinization.

(4.) Centrifuge at 500 × *g* for 3 min. Carefully remove the supernatant.

(5.) Resuspend the pellet in 2 mL cryo-preservation medium to a final concentration of about $0.8 \times 10^6$ $mL^{-1}$.

(6.) Aliquot 1 mL per vial into two cryovials. Label with water-resistant marker or deep-freeze label.

(7.) Freeze the suspension slowly overnight at −80 °C in a styrofoam box or controlled-rate freezing device. Transfer to liquid nitrogen the next day.

### T › *Thawing a cryo-preserved MCF 10A cell line*

(1.) Warm 20 mL growth medium to 37 °C; divide into two conical tubes.

(2.) *Critical:* Quickly thaw the cryogenic vial in a 37 °C water bath.

> ***Critical:*** Ensure the lid is tightly sealed and keep the vial upright to avoid contamination. Wipe thoroughly with disinfectant before transferring to a sterile environment.

(3.) Transfer thawed cells into one tube containing 10 mL pre-warmed medium.

(4.) Centrifuge at 500 × *g* for 3 min. Aspirate supernatant to remove DMSO.

(5.) Resuspend in remaining 10 mL of pre-warmed growth medium.

(6.) Plate the cells onto a 10 cm dish.

(7.) Incubate at 37 °C under 5% $CO_2$. Passage at P1 after 24–48 h. Begin experiments at P2 or later.

### D › *Cultivating MCF 10A in three-dimensional gels*

- ☐ Glass chamber slides, 8-well, pre-chilled
- ☐ Pipette tips, 200 µL, pre-chilled
- ☐ MCF 10A assay medium for 3D cultures
- ☐ Matrigel®

(1.) Thaw the required amount of Matrigel® at 4 °C. You will need 65 µL per well for the first week. Pre-chill pipette tips and chamber slides at −20 °C prior to use.

> ***Quality assurance:*** Each Matrigel® lot should meet the criteria outlined in the *Analysis* section! Failure to validate Matrigel® lots may compromise the reliability of experimental results.

(2.) Coat each well of an 8-well chamber slide by placing 40 µL of Matrigel® in the center of each well. Keep the chamber on ice and spread evenly using a new 200 µL pipette tip. Allow the gel to solidify at room temperature for 60–90 min. 60–90 min

(3.) Dissolve 8 µL Matrigel® in 200 µL assay medium for 3D cultures per well to make a 4% Matrigel® solution. Keep the solution on ice and store the remainder at 4 °C.

(4.) *Optional:* Remove cell debris by rinsing the well briefly with 0.05% trypsin/EDTA.

(5.) Dissociate MCF 10A cells with 0.05% trypsin/EDTA according to *Alternative A*. Gently triturate clumps by pipetting up and down at least 15 to 20 times in 2 mL assay medium for 3D cultures. Make up to 10 mL, then count and assess viability. 🕮

(6.) Prepare a single-cell suspension of $2.0\times10^4$ $mL^{-1}$ cells.

(7.) Per well, combine 200 µL of the single-cell suspension (4 000 cells) with the Matrigel® solution. 🕮

(8.) Transfer 400 µL cell suspension onto each Matrigel®-coated well. Incubate at 37 °C under 5% $CO_2$.

(9.) After 12–16 h, add 300 µL fresh assay medium supplemented with 6 µL Matrigel® (2% final concentration). Do not remove supernatant at this time. ☾

(10.) Feed the cells every four days. Very carefully remove any supernatant and add back 400 µL fresh assay medium supplemented with 2% Matrigel® during the first week:

| | **Week 1** | | | **Week 2** | | **Week 3** | |
|---|---|---|---|---|---|---|---|
| **Time** | **Day 1** | **Day 2** | **Day 5** | **Day 9** | **Day 13** | **Day 17** | **Day 21** |
| Schedule A | Mon | Tue | Fri | Mon | Fri | Tue | Mon |
| Schedule B | Thu | Fri | Mon | Thu | Wed | Mon | Thu |
| Action | **Seed** | Feed | Feed | Feed | Feed | Feed | STOP |
| Assay medium for 3D cultures | 400 µL | 300 µL | 400 µL | 400 µL | 400 µL | 400 µL | 400 µL |
| Matrigel® concentration | 2% | 2% | 2% | Omit | Omit | Omit | Omit |

(11.) Proceed with downstream assays. 🐞

## *List of references*

Y. Qu, B. Han, Y. Yu, W. Yao, S. Bose, B.Y. Karlan, A.E. Giuliano, and X. Cui, *PLoS One* **10**(7), e0131285 (2015).