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).

Risk assessment

– Work with human-derived material (BSL-2)
▷ Wear gloves, safety glasses, lab coat
□ Collect and dispose waste after inactivation as REGULATED MEDICAL WASTE

Reviewed: Mar 14, 2023

Briefly

○ Grow MCF 10A in assay medium based on DMEM/F-12 + 5% horse serum, supplemented with insulin, hydrocortisone, cholera toxin, and 20 µg/L EGF, at 37 °C under 5% CO_2 in 95% relative humidity.

Critical: To maintain EGF signaling, replace assay medium every 48 h.

Hint: Alternatively, use MEBM supplemented with 100 ng/mL cholera toxin or commercial MEGM medium.

○ *Optional:* Supplement 50–100 U/mL penicillin + 50–100 µg/mL streptomycin.

Hint: Avoid antibiotics and antimycotics for routine culture work. They may mask contamination and interfere with metabolism in sensitive cells.

○ Subculture at 70–80% confluence by passaging 1:3 to 1:4 every 72 h.

Critical: Do not maintain cultures longer than 10 to 15 passages or more than one month. MCF 10A cells do not show contact inhibition. They must be regularly passaged, and supplied with fresh EGF to maintain a homogenous growth pattern and metabolic state. Discard cultures that become over-confluent or display altered morphology.

Procedures

A › *Splitting adherent MCF 10A cells*

□ MCF 10A resuspension medium
□ 20 mg/L Epidermal growth factor, 100 µL ⟨*R*⟩
□ MCF 10A assay medium ⟨*R*⟩

(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.

> ***This is why:*** The cells will not attach well in the presence of trypsin.

(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 0.8×10^{6} mL^{-1}.

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

> ***Hint:*** One vial typically yields a 60% confluent 10 cm dish.

(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.

> ***Note:*** A Mr. Frosty™ Freezing Container can improve viability for sensitive lines.

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 ⟨*R*⟩
- ☐ 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

> ***Hint:*** For a 6-well plate, coat with 600 µL Matrigel® and add 100 000 cells in 1.5 mL assay medium containing 2% Matrigel®.

(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.

> ***Hint:*** Scale this step with the number of wells to seed. For a 8-well slide, dissolve 70 µL Matrigel® in 1.75 mL assay medium.

(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.

> ***Note:*** Count cells at a 1:10 dilution.

(6.) Prepare a single-cell suspension of 2.0×10^4 mL^{-1} cells.

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

> ***Note:*** Scale this step accordingly as needed.

(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.

Analyses

○ Matrigel® lots must be validated to ensure experimental consistency. Follow *Alternative D* and document the acini structures along the time-course:

- Phase contrast pictures from Day 1 to Day 20. More than 80% of acini structures should look round.
- Indirect immunofluorescence at the following stages:

Protein	**Antibody**				**Expected result**		
	Host	**Dil.**	**Company**	**Cat. No.**	**Day 6**	**Day 15**	**Day 20**
Ki67	Rb	1:50	Zymed	18-0191	>8 cells per acinus	<3 cells per acinus	<2 cells per acinus
Caspase-3	Rb	1:100	CST	9661S	Negative	Positive	Weak or negative
Laminin-5	Ms	1:200	Chemicon	MAB19562	Basal lamina	Basal lamina	Basal lamina
GM130	Ms	1:100	BD	610822	Apex	Apex	Apex
Lumen					No lumen	Cleared	Cleared

Quality assurance: Take pictures of the whole well and magnifications at all stages. Analyze the pictures on the same or the next day after the staining.

Troubleshooting

Freezing MCF 10A cells for long-term storage

In Step 7:

– Low viability after thawing frozen stocks
  - Freeze cells at high density ($> 2\times10^6$ /mL) in log phase. Stationary-phase cells survive cryopreservation poorly.
  - Ensure controlled-rate freezing. Direct placement at −80 °C without insulation can cause ice crystal damage.

Cultivating MCF 10A in three-dimensional gels

In Step 3:

– Matrigel polymerizes during dispensing
  - Keep all tubes and tips on ice. Pre-chill chamber slides at −20 °C. Work quickly. Matrigel gels irreversibly above 10 °C.

In Step 11:

– Acini are irregularly shaped or do not form lumens
  - Verify Matrigel lot quality per the Analysis section. Ensure EGF concentration is correct (5 µg/L for 3D, not the 20 µg/L used for 2D).
  - Check that single-cell suspension is properly dissociated. Clumps at seeding produce misshapen structures.

Recipes

MCF 10A resuspension medium

Amount	Ingredient	Stock	Final
400 mL	DMEM/F-12		
100 mL	Horse serum		20%

Use sterile liquids. ***Note:*** Obtain DMEM/F-12 and horse serum from Gibco™ (Cat. nos. 11330032, 16050122).

MCF 10A resuspension medium

Date: Sign:

Insulin, 10 g/L

Amount	Ingredient		Stock	Final
0.1 mL	Acetic acid, glacial	[64-19-7]	17.4 M	1%
100 mg	Insulin, from bovine pancreas	[11070-73-8]		10 g/L
To 10 mL	Water, reagent-grade, sterile			

Use sterile liquids; allow 15 min for reconstitution. Dispense into 500 µL aliquots. Store at −20 °C. ***Note:*** Obtain insulin from Sigma-Aldrich® (Cat. no. I1882).

10 g/L Insulin

1% Acetic acid, 10 g/L Insulin

Date: Sign: R0152

Hydrocortisone, 1 g/L

Amount	Ingredient		Stock	Final
20 mg	Hydrocortisone	[50-23-7]	362.46 g/mol	1 g/L
To 20 mL	Ethanol, absolute			

Use sterile liquids. Dispense into 500 µL aliquots. Store at −20 °C. ***Note:*** Obtain hydrocortisone from Sigma-Aldrich® (Cat. no. H0888).

1 g/L Hydrocortisone

DANGER

Reproductive toxicology

Date: Sign: R0153

Cholera toxin, 1 g/L

Amount	Ingredient		Stock	Final
0.5 mg	Cholera toxin	[9012-63-9]		1 g/L
To 0.5 mL	Water, reagent-grade, sterile			

Use sterile liquids. Dispense into 50 µL aliquots. Store at −20 °C. ***Note:*** Obtain cholera toxin from Sigma-Aldrich® (Cat. no. C8052).

1 g/L Cholera toxin

DANGER

Acute dermal toxicity

Date: Sign: R0154

Epidermal growth factor (EGF), 20 mg/L

Amount	Ingredient	Stock	Final
0.5 mg	Epidermal growth factor (EGF), human, recombinant		20 mg/L
To 25 mL	Water, reagent-grade		

Use sterile liquids; allow 15 min for reconstitution. Dispense into 200 µL aliquots. Store at −20 °C. ***Note:*** Obtain EGF from PeproTech® (Cat. no. AF-100-15).

20 mg/L Epidermal growth factor (EGF)

Date: Sign: R0155

MCF 10A assay medium

Amount	Ingredient		Stock	Final
500 mL	DMEM/F-12			
25 mL	Horse serum			5%
500 µL	Insulin	R0152	10 g/L	10 mg/L
250 µL	Hydrocortisone	R0153	1 g/L	0.5 mg/L
50 µL	Cholera toxin	R0154	1 g/L	0.1 mg/L

Store at 4 °C.

MCF 10A assay medium

5% Horse serum, 10 mg/L Insulin,
0.5 mg/L Hydrocortisone, 0.1 mg/L Cholera toxin

Date: Sign:

MCF 10A assay medium for 3D cultures

Amount	Ingredient		Stock	Final
500 mL	DMEM/F-12			
10 mL	Horse serum			2%
500 µL	Insulin	R0152	10 g/L	10 mg/L
250 µL	Hydrocortisone	R0153	1 g/L	0.5 mg/L
50 µL	Cholera toxin	R0154	1 g/L	0.1 mg/L

Store at 4 °C.

MCF 10A assay medium for 3D cultures

2% Horse serum, 10 mg/L Insulin,
0.5 mg/L Hydrocortisone, 0.1 mg/L Cholera toxin

Date: Sign:

Matrigel®

Amount	Ingredient	Stock	Final
60 µL	Matrigel®, growth factor reduced		

Thaw Matrigel® on ice; dispense 65 µL aliquots into pre-chilled 1.5 mL tubes; label tubes with the lot number. Store at −20 °C. ***This is why:*** Matrigel® is a hydrogel reconstituted from perlecan, laminin, collagen IV, and nidogen-1 ("entactin") which acts as crosslinker; the basement membrane components are extracted from Engelbreth-Holm-Swarm mouse sarcoma cells.

Matrigel®

Date: Sign:

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).

Change log

2015-03-15	Shany Koren-Hauer	Initial protocols for two-dimensional and three-dimensional culture
2023-03-14	Benjamin C. Buchmuller	Adaptation as SOP.