



Title: **WiCell Embryonal Carcinoma Cell Line GCT27DC1 Protocols**

Document type: **User Protocols**

Document ID: **SH-16**

Author: Jenna Gay

Owner: Jenna Gay

Approver(s): Jenna Gay
approved at 2020-09-29 15:06 (UTC -0500)
Jenny Brehm
approved at 2020-09-30 14:29 (UTC -0500)

Approval date: 2020-09-30

Effective date: 2020-10-07

1. Preface

This booklet of protocols is intended to serve as a primer for culturing GCT27DC1, an embryonal carcinoma (EC) cell line, on mouse embryonic fibroblasts (MEFs). These protocols are representative of how the cells were cultured and banked. WiCell recommends that GCT27DC1 should be first thawed and established in the conditions in which they were initially frozen prior to transfer to alternate culture platforms. We recommend that you read through these protocols prior to thawing your cells, and follow them until you have established your own cell bank of frozen vials. As you thaw and expand your initial cell vials, we strongly encourage you to establish your own bank of frozen vials as soon as possible. Once the culture is established, freeze back a portion of the material, and freeze again when you have enough cells for 20 vials. This will ensure you have an adequate stock of material to expand from as you conduct experiments.

Because this bank will be the basis of all future work with this cell line, WiCell recommends that it be screened for STR post thaw to ensure that the identity of the banked material is as expected. This and other tests are available from WiCell's full-service Characterization Laboratory; to learn more about WiCell Characterization's offerings and how to submit samples visit www.wicell.org/characterization.

If you have any additional questions, please contact us through technical support on the WiCell website at www.wicell.org. © 2020 WiCell ®

NOTE: Culture protocols for alternate embryonal carcinoma (EC) cell lines, such as GCT27C4, are very different from protocols detailed here and must be adhered to for success.

2. Protocols

Section 1: MEF Plating Density Recommendations

For MEF based culture systems, WiCell recommends using only CF1 strain MEFs. For Embryonal Carcinoma Cell Line GCT27DC1, the plating density for freshly inactivated MEFs is 5×10^4 cells/cm². If inactivated MEFs have been frozen and thawed before use, WiCell recommends plating at a density of 6×10^4 cells/cm² to compensate for the loss of viability following the freeze thaw process. There can be significant variation in MEF quality from lot to lot, and therefore WiCell recommends qualifying all MEFs used to assure that they will support cell co-culture before use with critical cell lines.

For MEFs to adhere properly, vessels must be coated with 0.1% gelatin. It is recommended that gelatin be allowed to coat plates for 24 hours in a 37°C incubator prior to use. In cases of extreme urgency, a minimum of one hour of coating time is required. Used plated MEFs within 10 days for maximum quality.

Section 2: Thawing Embryonal Carcinoma Cell Line GCT27DC1

Note: Before thawing, check the certificate of analysis included in the shipping packet insert to acquire the recommended number of wells one vial should be thawed into (this can vary among different lots). In general, ROCK inhibitor is not required for most cell lines. Please consult the Certificate of Analysis to see if ROCK inhibitor is recommended. If ROCK inhibitor is not recommended, it will not be mentioned on the Certificate of Analysis.

Required Equipment

1. Biosafety cabinet
2. 37°C / 5% CO₂ incubator
3. 37°C water bath
4. Centrifuge
5. Microscope

Required Supplies

1. Forceps
2. Cryogenic handling gloves and eye protection
3. 5ml and/or 10ml sterile serological pipettes (Fisher, 13-678-27E, 13-67827F) or equivalent
4. 95% Ethanol
5. 6-well plate with inactivated MEF cells (3-6 wells of MEFs), refer to Section 1
6. 15ml conical tubes (Corning, 430052)
7. 250ml Nalgene filter (Fisher Scientific, 09-741-04)
8. 6-well plates (Falcon, 08-772-1B or Nunc, 140675) [surface area of 1 well = 9.6cm²]

Required Reagents

1. Fetal bovine serum (FBS) (Peak Serum, PS-FB1) or equivalent
2. GlutaMAX supplement (ThermoFisher, 35050061)
3. Penicillin-Streptomycin (ThermoFisher, 15140-122) or equivalent
4. DMEM (liquid) (ThermoFisher, 11965092) or equivalent
5. **Embryonal Carcinoma (EC) Culture Medium** (may also be referred to as EC culture medium or CS medium) (250ml)
 1. EC Culture Medium is also referenced in sections 3, 4 and 5.
 2. To make EC Culture Medium combine following components, filter sterilize, and store at 4°C for up to 14 days. Scale up or down to suite your needs.
 3. 225ml DMEM
 4. 25ml FBS
 5. 2.5ml GlutaMAX
 6. 2.5ml Penicillin-Streptomycin

Remove Vial and Thaw

1. Prior to removing vial from storage, acquire the thaw recommendation (number of wells one vial should be thawed into) found in the certificate of analysis included in the shipping packet insert.
2. Wear eye protection as vials stored in liquid nitrogen may accidentally explode when warmed.
3. Wear ultra-low temperature cryo gloves. Remove the cell vial from the liquid nitrogen storage tank using forceps.
4. Immerse the vial in a 37°C water bath without submerging the cap. Swirl the vial gently.
5. When only a small ice crystal remains, remove the vial from the water bath.
6. Ensure the vial cap is tightly closed and immerse the vial into a 95% ethanol bath to sterilize the outside of the tube. Briefly (15-30 seconds) air-dry the vial in the sterile biosafety cabinet.

Removal of Cryoprotectant and Re-suspension of Pluripotent EC Cells

1. Transfer the cells gently into a sterile 15ml conical tube using a 1ml or 5ml glass pipette. Do not allow cells to flow down the side of the tube, as this will cause shearing and reduce attachment.
2. Slowly, add 10ml of warmed EC culture medium drop-wise to cells in the conical tube. While adding the

medium, gently move the tube back and forth to mix the pluripotent stem cells. This reduces osmotic shock to the cells.

3. Centrifuge the cells at 200 x g for 5 minutes.
4. Aspirate and discard the supernatant with a sterilized Pasteur Pipette.
5. Re-suspend the cell pellet in 2ml EC culture medium for every well that will receive cells (number of wells receiving cells is based on the thaw recommendation found in the certificate of analysis which is included in the shipping packet insert). *For example:* When the thaw recommendation is to thaw 1 vial into 1 well, re-suspend the pellet in 2ml.
6. Gently pipette cells up and down in the tube a few times.

Plate EC Cells

1. Label a 6-well plate containing inactivated MEF cells with the passage number from the vial, the date and your initials.
2. Aspirate the MEF medium from wells that will receive cells (number of wells determined by the thaw recommendation found in the certificate of analysis). Rinse the well with 1ml sterile DMEM. Aspirate the DMEM rinse medium prior to plating.
3. Slowly add the 2ml cell suspension into the well(s).
4. Place plate into the incubator and gently move the plate back and forth and side to side to evenly distribute the cells—avoid circular motions to prevent pooling in the center of the well.
5. The next day, remove the spent medium and gently add 2ml/well of EC culture medium.
6. Feed every other day until ready to passage or freeze.

Section 3: Feeding Embryonal Carcinoma Cell Line GCT27DC1

Equipment

1. Biosafety cabinet
2. 37°C / 5% CO₂ incubator
3. Microscope

Required Supplies

1. 5ml and/or 10ml sterile serological pipettes (Fisher, 13-678-27E, 13-67827F) or equivalent

Required Reagents

1. EC Culture Medium as in Section 2.

Feeding EC cells

1. Observe the EC cells using a microscope. If they require passaging, follow the passaging protocol below.
2. Warm enough medium to feed 2ml for each well that will be fed.
3. Aspirate the spent medium with a sterilized Pasteur pipette. If feeding more than one plate, use a different pipette for each plate to reduce risk of contamination.
4. Add 2ml/well. After pipettes are used once, they must be disposed to reduce the contamination potential. Do not reinsert a used pipette into sterile medium for any reason.
5. Return the cells to the 37°C incubator.
6. Feed every other day until ready to passage or freeze.

Section 4: Passaging Embryonal Carcinoma Cell Line GCT27DC1

Required Equipment

1. Biosafety cabinet
2. 37°C / 5% CO₂ incubator
3. 37°C water bath

Required Supplies

1. 5ml sterile serological pipettes (Fisher, 13-678-27E) or equivalent
2. 15ml conical tubes (Corning, 430052)
3. 6-well plate with inactivated MEF cells (3-6 wells of MEFs)
4. 50ml Nalgene filter (FisherScientific, 09-741-88)
5. 6-well plates (Falcon, 08-772-1B or Nunc, 140675) [surface area of 1 well = 9.6cm²]

Required Reagents

1. 6-well plate with inactivated MEF cells (3-6 wells of MEFs), refer to Section 1
2. Dispase (ThermoFisher, 17105-041)
3. EC Culture Medium as in section 2.
4. DMEM (liquid) (ThermoFisher, 11965092) or equivalent
5. DPBS without Calcium and Magnesium (PBS) (Fisher Scientific, 14-190-144)
6. **1mg/ml Dispase Solution**
 1. Dispase solution is also referenced in section 5.
 2. To make 1mg/ml Dispase Solution combine the following components, filter sterilize and store at 4°C for up to 14 days. Scale up or down based on your needs.
 3. 15mg Dispase
 4. 15ml DMEM

Prepare Plate to Receive Cells

1. Ready the prepared MEF plate for culture by aspirating the excess medium from the wells, and rinsing with 1ml/well DMEM.
2. Add 1ml of warmed EC culture medium to each well that will receive cells.
3. Label plate appropriately (WiCell recommends at minimum the cell line name, passage number, date and technician initials) and set aside in the biosafety cabinet until ready to use.

Passaging EC Cells

1. Place plate of cells in biosafety cabinet.
2. Aspirate EC culture medium and wash with 1ml/well PBS.
3. Aspirate PBS, add 1ml/well Dispase Solution.
4. Incubate at 37°C/5% CO₂ incubator for 5-10 minutes or until cells detach from plate as colonies.
 1. MEFs will stay attached to the plate.

2. This may take longer than 10 minutes depending on activity of dispase.
5. Add 1ml/well EC culture medium to the detached colonies and gently transfer to a 15ml conical tube.
6. Allow cells to settle and form a loose pellet for 5-10 minutes.
7. Aspirate supernatant, be very careful to not disturb the cell pellet. Alternately, a 5ml or 10ml glass pipet may be used to remove the supernatant.
8. Resuspend the pellet by gently adding 10ml of EC culture medium to wash away the dispase.
9. Allow cells to settle and form a loose pellet for 5-10 minutes.
10. Aspirate supernatant, being very careful to not disturb the cell pellet. Alternately, a 5ml or 10ml glass pipet may be used to remove the supernatant.
11. Add 1ml for every well that will receive cells.
 1. For example, if one well was split 1:3, add 3ml EC culture medium to the cell pellet.
12. Pipet up and down gently a few times to slightly break up the clumps.
13. Transfer 1ml of cell suspension into each prepared well.
14. Place plate into the incubator and gently move the plate back and forth and side to side to evenly distribute the cells—avoid circular motions to prevent pooling in the center of the well.
15. The next day, remove the spent medium and gently add 2ml/well of EC culture medium.
16. Feed every other day until ready to passage or freeze.

Section 5: Freezing Embryonal Carcinoma Cell Line GCT27DC1

Required Equipment

1. Biosafety cabinet
2. 37°C / 5% CO₂ incubator
3. -80°C Ultra low freezer
4. Microscope

Required Supplies

1. 5ml and/or 10ml sterile serological pipettes (Fisher Scientific, 13-678-27E, 13-67827F) or equivalent
2. 15ml conical tubes (Corning, 430052)
3. Isopropanol freezing containers, and isopropanol
4. Cryovials (Fisher Scientific, 03-337-7Y) or equivalent
5. Cryovial rack
6. Cryogenic handling gloves and eye protection
7. Metal forceps
8. Ice bucket, ice

Required Reagents

1. EC Culture Medium, as seen in section 2.
2. 1mg/ml Dispase Solution, as seen in section 4.

3. DPBS without Calcium and Magnesium (PBS) (Fisher Scientific, 14-190-144), or equivalent
4. Dimethyl Sulfoxide (DMSO) 10ml ampoules (Sigma Aldrich, D2438), or equivalent

Prepare for Freeze

1. Cells are to be frozen at 2 vials per 1 well of a 6-well plate, with a final total volume of 1 ml/vial.
2. Label cryovials with the cell line, passage number (increase the passage number on the plate by 1 to label the vial so that the passage number on the vial is reflective of the passage number at thaw), the freeze date, and your initials. Use an alcohol proof pen or labels that resist liquid nitrogen and ethanol. Place in biosafety cabinet.
3. Sterilize the biosafety cabinet (with the labeled vials in it) for 20 minutes with UV light. Turn on the blower and open the sash. Spray down the whole surface with ethanol and allow it to evaporate for 20 minutes prior to initiating cryopreservation.
4. Obtain a recharged, room-temperature isopropanol freezing container. The isopropanol must be replaced every 5 uses.

Harvest and Freeze cells

1. View all cells under the microscope. Discard any contaminated plates.
2. Aspirate EC culture medium and rinse with 1 ml/well PBS.
3. Aspirate PBS and add 1 ml/well Dispase solution.
4. Incubate in 37°C/5% CO₂ incubator for 5-10 minutes or until cells detach from plate as colonies. MEFs will stay attached to the plate. NOTE: This may take longer than 10 minutes.
5. Add 1 ml/well EC culture medium and transfer detached colonies to a conical tube.
6. Repeat harvest for any remaining plates. Continue to pool all cells into the same conical tube to create a uniform lot.
7. Let tube sit in the biosafety cabinet to allow cells to pellet by gravity for 5-10 minutes.
8. Aspirate the supernatant, taking extreme care not to aspirate the pellet. Alternatively, a 5ml or 10ml serological pipet may be used to remove the supernatant.
9. Using EC culture medium, gently resuspend the pellet in the equivalent to 1 ml/well harvested (if 12 wells were harvested, use 12ml EC culture medium) to wash away the dispase.
10. Again aspirate the supernatant, taking extreme care not to aspirate the pellet. Alternatively, a 5ml or 10ml serological pipet may be used to remove the supernatant.
11. Calculate the required volume of EC culture medium, which is equal to 90% of the final volume per vial (0.9ml/vial) x the number of vials. At a later step, the remainder volume (DMSO) will be added. See Table 1 for EC culture medium calculation examples.

Number of Wells Harvested	Number of Resulting Vials	ml of EC Culture Medium to use (0.9ml/vial)
3	6	5.4
6	12	10.8
12	24	21.6

24	48	43.2
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Table 1.

12. Put tube of cells on ice.
13. Freeze cells by working in batches of 18 vials (or fewer) because 18 vials is the capacity of one isopropanol freezing container.
14. Use a 10ml serological pipet to very gently pipet up and down to evenly mix the cell suspension. Transfer 16.2ml of cells to a new conical tube (0.9ml of cells per vial when 18 vials are being frozen).
15. Calculate the required volume of DMSO, which is 0.1ml/vial (10% of final volume). See Table 2 for DMSO calculation examples.

Number of Vials	ml of DMSO to use (0.1ml/vial)
6	0.6
12	1.2
18	1.8

Table 2.

16. While slowly and gently turning/mixing the conical tube, slowly add calculated DMSO volume down the side of the tube drop by drop.
17. Once all DMSO has been added, use the same 5ml or 10ml serological pipet to very gently pipet up and down to evenly mix the cell suspension.
18. With the same pipette, distribute 1ml of cell suspension to each prepared vial. Mix cell pool every 6-10 vials for even distribution.
19. Ensure all vial caps are tightened and quickly place cryovials into an isopropanol containing freezing container. Place the freezing containers in the -80°C freezer overnight.
20. Repeat freezing in batches until all cells are frozen.
21. Transfer cell vials to liquid nitrogen storage the following day.

3. Version History

Version	Change Description	Effective Date
A	CC01010, Document Initiation.	02/25/2020
1.0	CC-332, Transitioned SOP-SH-016 to Qualio as SH-16.	08/19/2020
2.0	CC-407, updated section formatting/numbering. Updated the cell line name to proper name by removing the decimal point.	See Qualio or Controlled Copy

4. Attachments

No attachments.