INSTRUCTIONS FOR USE

Product Name: Primary Fibroblast

Product Description

Fibroblasts are involved in normal growth, healing, wound repair, and the day-to-day physiological activities of every tissue and organ in the body. When associated with carcinomas, they have been implicated in tumor progression. Fibroblasts are isolated from freshly resected tumor, as well as fibroblasts from the normal tissue beyond the margin of the tumor. Cells are cryopreserved after a limited number of passages and are provided as frozen ampoules. All cases are provided with a standard set of clinical data.

Quality Control

The cells are grown in antibiotic-free medium and monitored for bacterial contamination. The cell cultures have tested mycoplasma negative.

Storage

One vial of cells in cryopreservation media (CryoStor® CS5, Sigma Aldrich). Store vials at ≥ -80° C or colder until ready to use.

Handling/Caution Statement

Use Biosafety Level 1 safety procedures when handling these primary cells.

Materials

Reagent	Recommended Supplier	Part Number
DMEM or refer to BioIVT website Cancer Cell Inventory List	Gibco	11965-092
FGM	Lonza	CC-3131
FGM BulletKit	Lonza	CC-3130
Fetal Bovine Serum (Qualified)	Gibco	26140-079
TrypLE™ Express (*or trypsin*)	Gibco	12605-010
HBSS, no calcium, no magnesium	Gibco	14170-112

Materials
Sterile Conical Tubes, 50mL
Cell Culture Treated Flask, T-25
Cell Culture Treated Flask, T-75
70% Ethanol (EtOH)

Equipment
Biological Safety Cabinet (BSC)
Centrifuge
Incubator: 37°C, 5% CO_2
Water bath, 37°C

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Protocol

Culture Medium Preparation

Culture Medium:

1. In Biological Safety Cabinet (BSC):
    a. Refer to the BioIVT website Cancer Cell inventory list to select the appropriate lot specific Culture Medium.
    b. Prepare reagents (refer to manufacturer instructions for recommended protocols).
    c. Prepare the Culture Medium according to the recipe listed in Table 1 below.
2. Store prepared Culture Medium at 2-8°C until ready for use.

Table 1: Primary Fibroblast Culture Medium

Component	Stock Concentrations	Final Concentrations	Amount added to 500ml
DMEM or FGM	-	-	450ml (for 10% FBS) or 475ml (for 5% FBS) bottle
Fetal Bovine Serum (Qualified), **heat-inactivated**	-	5% or	25ml
Fetal Bovine Serum (Qualified), **heat-inactivated**	-	10%	50ml

Or

FGM BulletKit

Antibiotic/Antimycotic usage

BioIVT does not recommend the use of antibiotics or antimycotics. Use in cell culture media at your own discretion.

Cell Thaw

Note: *Some liquid nitrogen-stored vials may blow off cap when transferred to warm water due to gas overexpansion. Always wear appropriate PPE when handling frozen vials and perform the following steps as directed.*

3. Equilibrate Culture Medium to 37°C.
4. In BSC, transfer 25ml of Culture Medium to a 50ml conical tube.
5. Using sterile technique, twist vial cap one quarter turn. Re-tighten cap.
6. Quickly swirl and thaw vial in 37°C water bath (~ 2 minutes). Do not submerge vial past cap threads. Immediately remove vial from bath the moment thaw is complete. **Do not allow the suspension to warm**.
7. Disinfect vial with 70% ethanol (or equivalent) and place in BSC.

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Cell Culture

8. In BSC:
    a. Quickly transfer thawed contents from cell vial into the 50ml conical containing pre-warmed Culture Medium and rinse pipette tip 3-5 times.
    b. Optional: rinse vial with Culture Medium to collect any remaining cells and transfer to 50ml conical.
    c. Mix entire suspension thoroughly.
9. Centrifuge the cell suspension at approximately 200xg for 5-10 minutes.
10. After centrifugation is complete, transfer conical tube to BSC:
    a. Remove supernatant.
    b. Re-suspend cells with 2-3ml of pre-warmed Culture Medium.
    c. Remove sample for counting and viability testing (approximately 50µl).
    d. Transfer cell suspension to appropriately sized culture flask and add media as indicated:

Table 2: Cell seeding volume

Flask	Volume	Total # Cells
T-25	5ml	$4x10^5$
T-75	15ml	$1x10^6$

11. Gently rock the culture flasks to evenly distribute the cells.
12. Place flask into a 37°C incubator at 5% CO_2.
13. Incubate for 2 days then perform **Cell Maintenance** steps.

Cell Maintenance

14. Equilibrate Culture Medium to 37°C.
15. Evaluate cells to confirm attachment to culturing vessel and to determine percent confluency (refer to Appendix for visual reference).

    Note: *Cells do not grow well at low density and should be subcultured at higher densities (see Table 2).*

    a. If cells are less than 90% confluent, perform steps 16-19.
    b. If cells are at least 90% confluent, proceed to **Cell Subculturing** steps.
16. In BSC:
    a. Remove supernatant.
    b. Add appropriate amount of warmed Culture Medium (refer to Table 2).
17. Place flask into a 37°C incubator at 5% CO_2.
18. Incubate cells; observe daily and repeat **Cell Maintenance** steps as necessary.
19. Change media 3 times per week for established cultures.

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Cell Subculturing

20. Equilibrate Culture Medium, TrypLE™ and serum-free isotonic solution without calcium or magnesium (e.g. HBSS -/- or equivalent) to 37°C.
21. In BSC:
    a. Remove supernatant.
    b. Rinse flask with 3-5ml serum-free isotonic solution and discard.
    c. Add 2-5ml fresh, pre-warmed TrypLE™.
22. Incubate at 37°C, checking for cell dissociation every 2 minutes, until cells are detached.
23. Once cells have detached, transfer flask to BSC and add a volume of Culture Medium equal to that of the TrypLE™ used (to neutralize TrypLE™).
24. Aspirate and pipette cell suspension several times to obtain a single-cell suspension.
25. Transfer the suspension to a 50ml conical tube.
26. Rinse the flask with an additional 3-5ml of pre-warmed Culture Medium to collect residual cells.
27. Pipette and thoroughly mix the suspension in the conical tube.
28. Perform **Cell Culture** steps 9-13.
    a. Passage cells every 2-4 days at 1:3 to 1:5 split ratio.
    b. Refer to Table 2 for cell seeding volumes.

Freezing Cells

29. Place a controlled rate freezing unit (e.g. Nalgene® Mr. Frosty) at 4°C 1-2 hours prior to expected usage.
30. Perform **Cell Subculturing** steps 20-27.
31. Proceed to perform **Cell Culture** steps 9 & 10a-c.
32. When cell counts/ml of suspension has been determined, centrifuge the suspension again at 200xg for 5-10 minutes.
33. After centrifugation is complete, transfer conical tube to BSC.
    a. Remove supernatant.
    b. Gradually add cooled (4°C) cryopreservation medium (CryoStor® 5 or preferred cryopreservation medium) to re-suspend the pelleted cells to the desired concentration.
34. Mix to a homogenous suspension and aliquot to cryopreservation vials.
35. Transfer vials to the pre-cooled controlled rate freezing unit.
36. Place the controlled rate freezing unit in -80°C freezer for 24 hours.
37. Transfer vials of cells from controlled rate freezing unit to liquid nitrogen vapor phase or -80°C storage.

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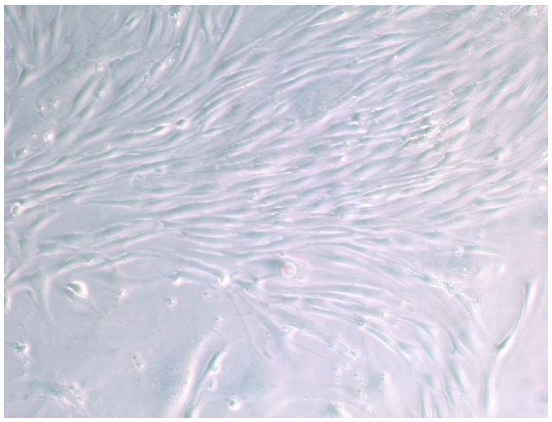
Troubleshooting

Problem	Probable Cause	Solution
▸ Poor cell growth ▸ Poor attachment	• Media incorrectly supplemented • Cell plate density too low • Cell confluence too high before splitting • Incubator temperature / CO2 settings	▸ Ensure media supplements are reconstituted per vendor instructions ▸ Plate cells at recommended density (Table 2) ▸ Passage cells at 1:3 split ratio ▸ Incubate at 37°C / 5% CO2 ▸ Increase FBS in Culture Medium from 5% to 10% until suitable attachment is observed
Low viability	• Vial thaw procedure error (vial left in warm water bath too long) • Vial storage temperature too high	▸ Perform quick thaw procedure ▸ Store vial at -70°C or lower prior to thaw

Appendix

Picture 1: Stromal fibroblasts



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