INSTRUCTIONS FOR USE

Product Name: SUM102PT

Product Description

The SUM102PT cell line was developed from a patient with minimally invasive, ER negative, PR negative and Her2 positive human breast carcinoma. The cell line is a representative of a class of human breast cancers characterized by a high level of EGFR expression in the absence of gene amplification.

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 1 x 10^6 cells in cryopreservation media (CryoStor® CS5, BioLife Solutions).

Handling/Caution Statement

Use Biosafety Level 1 safety procedures when handling this cell line.

Materials

Reagent	Recommended Supplier	Part Number
Ham's F-12	Gibco	11765
Phosphate Buffered Saline (PBS) or other serum-free isotonic solution	Gibco	10010031
Bovine Serum Albumin (fatty acid free)†	Sigma-Aldrich	A8806
Epidermal Growth Factor (EGF)*	Sigma-Aldrich	E9644
Ethanolamine	Sigma-Aldrich	E0135
Fetal Bovine Serum (Qualified)‡, **heat-inactivated**	Gibco	26140-079
HEPES	Sigma-Aldrich	H3375
Hydrocortisone (*Do not sterile filter*)	Sigma-Aldrich	H4001
Insulin	Sigma-Aldrich	I9278
Sodium Selenite (Se)	Sigma-Aldrich	S9133
apo-Transferrin	Sigma-Aldrich	T2252
Triiodo-L-Thyronine (T3)	Sigma-Aldrich	T5516
TrypLE™ Express (*or trypsin*)	Gibco	12605-010
CryoStor CS5	BioLife Solutions	205102

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* It is highly recommended that only recombinant EGF from Sigma-Aldrich be used in the culture of SUM102PT.
†It is highly recommended that only BSA from Sigma-Aldrich be used in the culture of SUM102PT.
‡FBS is used for the initial 24 hours of culture (from frozen stock or subculturing).

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

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

Protocol

Culture Medium Preparation

Note: *This cell line requires 2 different media formulations, "Culture Medium" and "2% FBS Culture Medium". Use the media with FBS when thawing and subculturing cells into new culture vessels to ensure that they attach. Once the cells are well attached switch to the Culture Medium (without FBS) for regular media changes.*

Culture Medium

Used for Cell Maintenance and preparation of **2% FBS Culture Medium** for Cell Thaw, Post Thaw Culture, and Cell Subculturing.

1. In Biological Safety Cabinet (BSC)
   a. Prepare reagents (refer to manufacturer instructions for recommended protocols).
   b. Prepare *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: SUM102PT *Culture Medium*

Component	Stock Concentrations	Final Concentrations	Amount added to 500ml
Ham's F-12	-	-	467ml
Bovine Serum Albumin (fatty acid free)*	20g/L	1g/L	25ml
Epidermal Growth Factor (EGF)	10µg/ml	10ng/ml	500µl
Ethanolamine	16.6M	5mM	151µl
HEPES	1M	10mM	5ml
Hydrocortisone	1mg/ml	1µg/ml	500µl
Insulin	1mg/ml	5µg/ml	2.5ml
Sodium Selenite (Se)	20µg/ml	8.7ng/ml (50nM)	216µl
apo-Transferrin	2.5mg/ml	5µg/ml	1ml
Triiodo-L-Thyronine (T3)	20µg/ml	6.7ng/ml (10nm)	168µl

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2% FBS Culture Medium:

Used for Cell Thaw, Post Thaw Culture, and Cell Subculturing

3. In Biological Safety Cabinet (BSC):
   a. Transfer 49ml of prepared *Culture Medium* into a 50ml conical tube (or equivalent).
   b. Add 1ml of Fetal Bovine Serum (FBS) to 50ml conical tube.
4. Warm supplemented medium in 37°C water bath prior to use.

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 protective clothing when handling frozen vials and perform the following steps as directed.*

5. Equilibrate **2% FBS Culture Medium** to 37°C.
6. In BSC, transfer 25ml of **2% FBS Culture Medium** to a 50ml conical tube.
7. Using sterile technique, twist cell vial cap one quarter turn. Re-tighten cap.
8. 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.**
9. Disinfect vial with 70% ethanol (or equivalent) and place in BSC.

Post Thaw Culture

10. In BSC:
    a. Quickly transfer thawed contents from cell vial into the 50ml conical containing pre-warmed **2% Culture Medium** and rinse pipette tip 3-5 times.
    b. Optional: rinse vial with **2% Culture Medium** to collect any remaining cells and transfer to 50ml conical.
    c. Mix entire suspension thoroughly.
11. Centrifuge the cell suspension at approximately 200xg for 5-10 minutes.
12. After centrifugation is complete, transfer conical tube to BSC:
    a. Remove supernatant.
    b. Re-suspend cells with 2-3ml of pre-warmed **2% Culture Medium**.
    c. Remove sample for counting and viability testing (approximately 20µl).
    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	$1x10^6$

13. Gently rock the culture flasks to evenly distribute the cells.

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14. Place flask into a 37°C incubator at 5% CO2.
15. Incubate for 1 day then perform Post-seeding Evaluation.

Post-seeding Evaluation

16. The day after initiating cell culture, evaluate the cell adherence:
    - If the majority of cells are adherent, proceed with Cell Maintenance.
    - If there is poor adherence, transfer supernatant to a 50ml conical tube and repeat Post Thaw Culture steps 11 - 15 using **2% FBS Culture Medium**. Add cells to a new appropriately sized flask.

Cell Maintenance

17. Equilibrate *Culture Medium* to 37°C.
18. Evaluate cell confluence (refer to Appendix Picture 1 for visual reference).

> **Note**: *Cells do not grow well at low density or beyond 90-95% confluence. Cultures typically reach confluence in approximately 7-10 days.*

    a. If cells are less than 90% confluent, perform steps 12 - 14.
    b. If cells are at least 90% confluent, proceed to Cell Subculturing steps.
19. In BSC:
    a. Remove supernatant.
    b. Add appropriate amount of warmed *Culture Medium* (refer to Table 2).
20. Place flask into a 37°C incubator at 5% CO2.
21. Incubate cells; observe daily and repeat Cell Maintenance steps as necessary.
22. Change media 3 times per week for established cultures.

Cell Subculturing

23. Equilibrate **2% FBS Culture Medium**, TrypLE™ and serum-free isotonic solution (e.g. phosphate buffered saline or equivalent) to 37°C.
24. In BSC:
    a. Remove supernatant.
    b. Rinse flask with 3-5ml pre-warmed serum-free isotonic solution and discard.
    c. Add 2-5ml fresh, pre-warmed TrypLE™.
25. Incubate at 37°C, checking for cell dissociation every 2 minutes, until cells are detached.
26. Once cells have detached, transfer flask to BSC and add a volume of pre-warmed **2% FBS Culture Medium** equal to that of the TrypLE™ used (to neutralize TrypLE™).
27. Aspirate and pipette cell suspension a number of times to obtain a single-cell suspension.
28. Transfer the suspension to a 50ml conical tube.
29. Rinse the flask with an additional 3-5ml of pre-warmed **2% FBS Culture Medium** to collect residual cells.
30. Pipette and thoroughly mix the suspension in the conical tube.
31. Perform Post Thaw Culture steps 11- 15.

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a. Passage cells every 7-10 days at 1:3 split ratio for the first one to three passages and up to 1:6 thereafter.
b. Refer to Table 2 for cell seeding volumes.

Freezing Cells

32. Place a controlled rate freezing unit (eg. Nalgene® Mr. Frosty) at 4°C 1-2 hours prior to expected usage.
33. Perform Cell Subculturing steps 23 - 30.
34. Proceed to perform Post Thaw Culture steps 11 & 12(a-c).
35. When cell counts/ml of suspension has been determined, centrifuge the suspension again at 200xg for 5-10 minutes.
36. 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.
37. Mix to a homogenous suspension and aliquot to cryopreservation vials.
38. Transfer to the pre-cooled rate freezing unit.
39. Place the controlled rate freezing unit in -80°C freezer for 24 hours.
40. Transfer vials of cells from -80°C to liquid nitrogen vapor phase.

Reference

F Forozan, R Veldman, CA Ammerman, NZ Parsa, A Kallioniemi, O-P Kallioniemi and SP Ethier. Molecular cytogenetic analysis of 11 new breast cancer cell lines. British Journal of Cancer (1999) 81(8), 1326-1334. DOI: 10.1038/sj.bjc.6695007

Troubleshooting

Problem	Probable Cause	Solution
♦ Poor cell growth ♦ Poor attachment	• Media incorrectly supplemented	▸ Ensure media supplements are reconstituted per vendor instructions
	• Cell plate density too low	▸ Plate cells at recommended density (Table 2) ▸ Use media with 2% FBS for plating cells
	• Cell confluence too high before splitting	▸ Passage cells at 1:3 split ratio
	• Incubator temperature / CO2 settings	▸ Incubate at 37°C / 5% CO2

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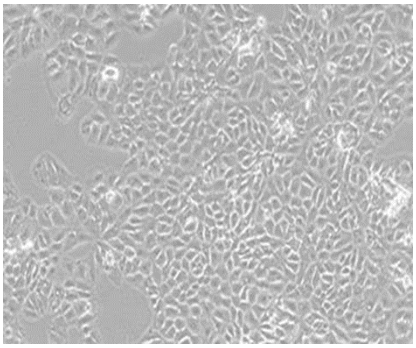
Low viability	• Vial thaw procedure error (vial left in warm water bath too long) • Vial storage temperature too high	▸ Perform quick thaw procedure ▸ Store vial at -70°C or lower prior to thaw

Appendix



Picture 1: SUM102PT cells in culture

Related Products

SUM44PE	SUM52PE
SUM149PT	SUM225CWN
SUM159PT	SUM229PE
SUM185PE	SUM1315MO2
SUM190PT	Primary Human Breast Cancer Associated Fibroblasts
Primary Human Breast Cancer Associated Epithelial Cells	

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