INSTRUCTIONS FOR USE

Product Name: SUM225CWN

Product Description

The SUM225CWN cell line was derived from a chest wall recurrence in an ER negative, PR negative and Her2 positive (amplified) patient that was diagnosed and treated six years previously for ductal carcinoma in situ. The chest wall specimen was positive for adenocarcinoma. The cell line is immortal and expresses luminal cytokeratins 8, 18, and 19 consistent with their origin from luminal breast epithelial cells.

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
Fetal Bovine Serum (Qualified)	Gibco	26140-079
HEPES	Sigma-Aldrich	H3375
Hydrocortisone (*Do not sterile filter*)	Sigma-Aldrich	H4001
Insulin	Sigma-Aldrich	I9278
TrypLE™ Express (*or trypsin*)	Gibco	12605-010
CryoStor CS5	BioLife Solutions	205102

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

North America & Asia Pacific
516-483-1196
customerservice@bioivt.com
PO Box 770, Hicksville NY 11802-0770,U.S.A.

Europe, Middle East & Africa
44 (0) 1444 250010
cseurope@bioivt.com
West Sussex RH15 9TN, U.K.

www.bioivt.com

Protocol

Culture Medium Preparation

Culture Medium

1. In Biological Safety Cabinet (BSC)
   a. Prepare reagents (refer to manufacturer instructions for recommended protocols).
   b. 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: SUM225CWN Culture Medium

Component	Stock Concentrations	Final Concentrations	Amount added to 500ml
Ham's F-12	-	-	469ml
Fetal Bovine Serum (Qualified), **heat-inactivated**	-	5%	25ml
HEPES	1M	10mM	5ml
Hydrocortisone	1mg/ml	1µg/ml	500µl
Insulin	10mg/ml	5µg/ml	250µl

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.

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 cell 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.

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.

North America & Asia Pacific
516-483-1196
customerservice@bioivt.com
PO Box 770, Hicksville NY 11802-0770,U.S.A.

Europe, Middle East & Africa
44 (0) 1444 250010
cseurope@bioivt.com
West Sussex RH15 9TN, U.K.

www.bioivt.com

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 20µl).
    d. Transfer cell suspension to appropriately sized culture flask and add media as indicated:

    Note: *Cells can be slow to recover and should be cultured at higher densities for several passages after thaw (e.g. 1:2 split)*

    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% CO2.
13. Incubate for 1 day then perform Cell Maintenance steps (below).

Cell Maintenance

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

> **Note**: *Cells are slow to recover from cryopreservation. Cells grow in clumps and generally do not reach 100% confluence. The cells typically reach maximum confluence in approximately 10-14 days.*

    a. If cells are less than 80% confluent, perform steps 16 - 18.
    b. If cells are at least 80-90% 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% CO2.
18. Incubate cells; observe daily and repeat Cell Maintenance steps as necessary.
19. Change media 3 times per week for established cultures.

Cell Subculturing

20. Equilibrate Culture Medium, TrypLE™ and serum-free isotonic solution (e.g. phosphate buffered saline or equivalent) to 37°C.
21. 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™.
22. Incubate at 37°C, checking for cell dissociation every 2 minutes, until cells are detached.

North America & Asia Pacific
516-483-1196
customerservice@bioivt.com
PO Box 770, Hicksville NY 11802-0770,U.S.A.

Europe, Middle East & Africa
44 (0) 1444 250010
cseurope@bioivt.com
West Sussex RH15 9TN, U.K.

www.bioivt.com

23. 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™).
24. Aspirate and pipette cell suspension a number of 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 7-10 days from 1:2 up to 1:3 split ratio.
    b. Refer to Table 2 for cell seeding volumes.

Freezing Cells

29. Place a controlled rate freezing unit (eg. 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 & 10(a-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® CS5 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 to the pre-cooled rate freezing unit.
36. Place the controlled rate freezing unit in -80°C freezer for 24 hours.
37. 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)

North America & Asia Pacific
516-483-1196
customerservice@bioivt.com
PO Box 770, Hicksville NY 11802-0770,U.S.A.

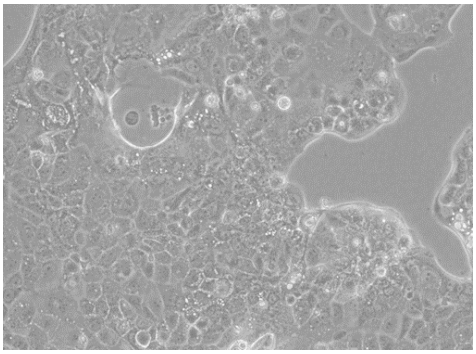
Europe, Middle East & Africa
44 (0) 1444 250010
cseurope@bioivt.com
West Sussex RH15 9TN, U.K.

www.bioivt.com

	• Cell confluence too high before splitting • Incubator temperature / CO2 settings	▸ Passage cells at 1:2 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

SUM225 cells can be slow to recover from the freeze/thaw process; best practice is to maintain the cells at higher densities for the first few passages. For example, start 1 vial in a T-25 flask and then split 1:2 until they are growing well and then passage them into a T-75. This should only take a few passages. These cells will generally not come to 100% confluency. As a result, these cells should be split at what looks like 50-60% confluent. The image below is a representation of what the cells should look like when they are ready to be split.

Appendix



Picture 1: SUM225CWN cells in culture

Related Products

SUM44PE	SUM52PE
SUM102PT	SUM190PT
SUM149PT	SUM229PE
SUM159PT	SUM1315MO2
SUM185PE	Primary Human Breast Cancer Associated Fibroblasts
Primary Human Breast Cancer Associated Epithelial Cells	

North America & Asia Pacific
516-483-1196
customerservice@bioivt.com
PO Box 770, Hicksville NY 11802-0770,U.S.A.

Europe, Middle East & Africa
44 (0) 1444 250010
cseurope@bioivt.com
West Sussex RH15 9TN, U.K.

www.bioivt.com