

Important: Please read these instructions carefully

The viability of these cells is warranted for 30 days from date of shipment when specified reagents and growth conditions are followed as described in this instruction.

If you feel there is a problem with this product, contact Asterand at 1-313-263-0960, 1-866-384-7783 (US Toll Free), +44 (0)1763 211600 (UK), or your sales representative.

These cells are for research use only. Please allow the cells to acclimate before use.

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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.

Contents and Storage

One vial of 1 x 10⁶ cells in cryopreservation media (CryoStor[®] CS5, BioLife Solutions).

Handling Procedures

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

Required Materials

Reagent	Recommended Supplier	Part Number
Ham's F-12	Gibco	11765
Fetal Bovine Serum (Qualified)	Gibco	26140-079
HEPES	Sigma-Aldrich	H3375
Hydrocortisone (<i>do not sterile filter</i>)	Sigma-Aldrich	H4001
Insulin	Sigma-Aldrich	I9278
TrypLE™ Express (<i>or trypsin</i>)	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

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: Asterand 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.

1. Equilibrate Culture Medium to 37°C.
2. In BSC, transfer 25ml of Culture Medium to a 50ml conical tube.
3. Using sterile technique, twist cell vial cap one quarter turn. Re-tighten cap.
4. 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.**
5. Disinfect vial with 70% ethanol (or equivalent) and place in BSC.

Cell Culture

1. 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.
2. Centrifuge the cell suspension at approximately 200xg for 5-10 minutes.
3. 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

4. Gently rock the culture flasks to evenly distribute the cells.
5. Place flask into a 37°C incubator at 5% CO₂.
6. Incubate for 1 day then perform Cell Maintenance steps (below).

Cell Maintenance

1. Equilibrate Culture Medium to 37°C.
2. Evaluate cell confluence (refer to Appendix A Cell Culture Images 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 3-5.
 - b. If cells are at least 80-90% confluent, proceed to Cell Subculturing steps.
3. In BSC:
 - a. Remove supernatant.
 - b. Add appropriate amount of warmed Culture Medium (refer to Table 2).
4. Place flask into a 37°C incubator at 5% CO₂.
5. Incubate cells; observe daily and repeat Cell Maintenance steps as necessary.
6. Change media 3 times per week for established cultures.

Cell Subculturing

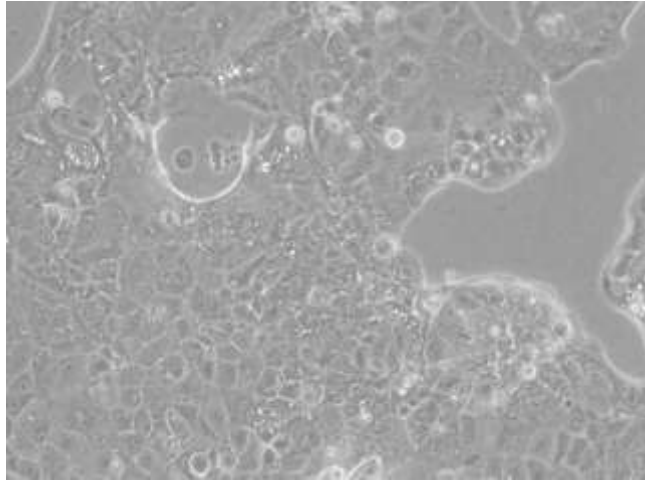
1. Equilibrate Culture Medium, TrypLE™ and serum-free isotonic solution (e.g. phosphate buffered saline or equivalent) to 37°C.
2. In BSC:
 - a. Remove supernatant.
 - b. Rinse flask with 3-5ml pre-warmed serum-free isotonic solution and discard.
 - c. Add 2-5ml fresh, pre-warmed TrypLE™.
3. Incubate at 37°C, checking for cell dissociation every 2 minutes, until cells are detached.
4. Once cells have detached, transfer flask to BSC and add a volume of pre-warmed Culture Medium equal to that of the TrypLE™ used (to neutralize TrypLE™).
5. Aspirate and pipette cell suspension a number of times to obtain a single-cell suspension.
6. Transfer the suspension to a 50ml conical tube.
7. Rinse the flask with an additional 3-5ml of pre-warmed Culture Medium to collect residual cells.
8. Pipette and thoroughly mix the suspension in the conical tube.
9. Perform Cell Culture steps 2-6.
 - a. Passage cells every 7-14 days from 1:2 up to 1:3 split ratio.
 - b. Refer to Table 2 for cell seeding volumes.

Freezing Cells

1. Place a controlled rate freezing unit (eg. Nalgene® Mr. Frosty) at 4°C 1-2 hours prior to expected usage.
2. Perform Cell Subculturing steps 1-8.
3. Proceed to perform Cell Culture steps 2 & 3 (a-c).
4. When cell counts/ml of suspension has been determined, centrifuge the suspension again at 200xg for 5-10 minutes.
5. 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.

6. Mix to a homogenous suspension and aliquot to cryopreservation vials.
7. Place vials on wet ice for 5-10 minutes then transfer to the pre-cooled controlled rate freezing unit.
8. Place the controlled rate freezing unit in -80°C freezer for 24 hours.
9. Transfer vials of cells from -80°C to liquid nitrogen vapor phase.

APPENDIX A: Cell Culture Images



Picture 1: SUM225CWN cells in culture