SUM190PT Instructions For Use

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.

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

Table of Contents

- Product Description
- Handling Procedures
- Required Materials
- Culture Medium Preparation
- Cell Thaw
- Cell Culture
- Post-seeding Evaluation
- Cell Maintenance
- Cell Subculturing
- Cell Culture Images

Product Description

The SUM190PT cell line was developed from a primary tumor from a patient with ER negative, PR negative and Her2 positive (amplified) breast cancer. The cell line is immortal and expresses luminal cytokeratins 8, 18, and 19 consistent with their origin from luminal breast epithelial cells. SUM190PT has been known to form tumors in nude mice.

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 10^6 cells in cryopreservation media (CryoStor® CS5, Sigma Aldrich).

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
Bovine Serum Albumin (fatty acid free)*	Sigma-Aldrich	A8806
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 Thyronine (T_3)	Sigma-Aldrich	T5516
TrypLE™ Express (*or trypsin*)	Gibco	12605-010
CryoStor CS5	BioLife Solutions	205102

* **It is highly recommended that only BSA from Sigma-Aldrich be used in the culture of SUM190PT.**

North America & Asia Pacific
614-245-0056
customerservice@bioivt.com
Detroit, MI 48202, U.S.A

Europe, Middle East & Africa
44 1763 211555
cseurope@bioivt.com
Royston SG8 5HD, U.K.

www.bioivt.com

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

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: SUM190PT culture medium

Component	Stock Concentrations	Final Concentrations	Amount added to 500ml
Ham's F-12	-	-	468ml
Bovine Serum Albumin	20g/L	1g/L	25ml
Ethanolamine	16.6M	5mM	151µl
HEPES	1M	10mM	5ml
Hydrocortisone	1mg/ml	1µg/ml	500µl
Insulin	10mg/ml	5µg/ml	250µl
Sodium Selenite (Se)	20µg/ml	8.7ng/ml (50nM)	216µl
apo-Transferrin	2.5mg/ml	5µg/ml	1ml
Triiodo Thyronine (T_3)	20µg/ml	6.7ng/ml (10nM)	168µl

Antibiotic/Antimycotic usage: Asterand does not recommend the use of antibiotics or antimycotics. Use in cell culture media at your own discretion.

2% FBS Culture Medium: Used for **Cell Thaw**, **Cell Culture** and **Cell Subculturing**

1. 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.
2. Warm supplemented medium in 37°C water bath prior to use.

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 2% FBS Culture Medium to 37°C.
2. In BSC, transfer 25ml of 2% FBS Culture Medium to a 50ml conical tube.
3. Using sterile technique, twist 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.

North America & Asia Pacific
614-245-0056
customerservice@bioivt.com
Detroit, MI 48202, U.S.A

Europe, Middle East & Africa
44 1763 211555
cseurope@bioivt.com
Royston SG8 5HD, U.K.

www.bioivt.com

Cell Culture

1. In BSC:
    a. Quickly transfer thawed contents from cell vial into the 50ml conical containing pre-warmed 2% FBS Culture Medium and rinse pipette tip 3-5 times.
    b. Optional: rinse vial with 2% FBS 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 2% FBS 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	$4x10^5$ cells
T-75	15ml	$1x10^6$ cells

4. Gently rock the culture flasks to evenly distribute the cells.
5. Place flask into a 37°C incubator at 5% CO_2.
6. Monitor for 24 hours then perform Post-seeding Evaluation.

Post-seeding Evaluation

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 Cell Culture steps 2-6 using 2% FBS Culture Medium. Add cells to a new appropriately sized flask.

Cell Maintenance

1. Equilibrate Culture Medium to 37°C.
2. Evaluate cell confluence (refer to Appendix A Cell Culture Images for visual reference).
   Note: *Cell line grows in clumps and generally will not reach 100% confluence*
    a. If cells are less than 50% confluent, perform steps 3-5.
    b. If cells are at least 50-60% confluent, perform Cell Subculturing steps. Cells generally require subculturing every 14-21 days.
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% CO_2.
5. Incubate cells; observe daily and repeat Cell Maintenance steps as necessary.
6. Change media 3 times per week for established cultures.

North America & Asia Pacific
614-245-0056
customerservice@bioivt.com
Detroit, MI 48202, U.S.A

Europe, Middle East & Africa
44 1763 211555
cseurope@bioivt.com
Royston SG8 5HD, U.K.

www.bioivt.com

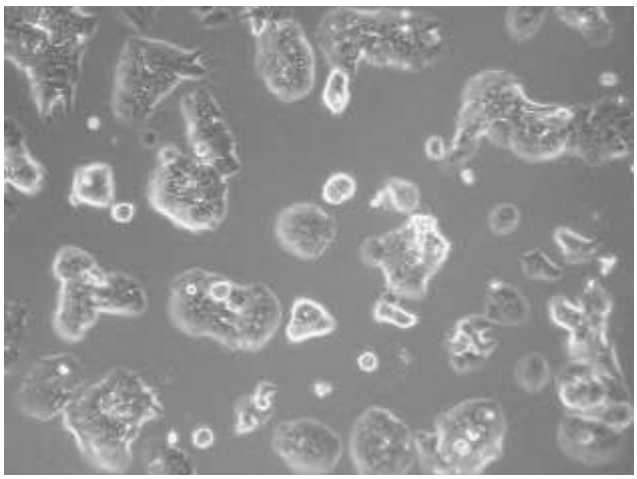
Cell Subculturing

1. Equilibrate 2% FBS 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 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 2% FBS 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 and Post-seeding Evaluation.
    a. Passage cells every 14-21 days at 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: SUM190PT cells in culture.

North America & Asia Pacific
614-245-0056
customerservice@bioivt.com
Detroit, MI 48202, U.S.A

Europe, Middle East & Africa
44 1763 211555
cseurope@bioivt.com
Royston SG8 5HD, U.K.

www.bioivt.com