Prostate Cell Line 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.

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Product Description

OPCT (prostate tumor) and **OPCN** (prostate normal) epithelial cell lines were derived from tumor and adjacent normal tissues obtained (in sets from the same patient) at the time of prostatectomy. The cell lines were immortalized via replication-defective retrovirus transferring the transforming HPV16 or HPV18 E6 and E7 genes, as well as *neo*^{R}, however they are not clonal.

All OPC lines are positive for prostate stem cell antigen (PSCA), vimentin and cytokeratins 5, 8 and 18, indicating their prostate epithelial cell origin. They are negative for desmin, alpha smooth muscle actin (αSMA) and factor 8.

OPCT cell lines are derived from less advanced primary tumor than some other commercially available metastatic cell lines (e.g. PC-3, LNCaP, DU 145).

Quality Control
The cells are grown in antibiotic-free medium and monitored for bacterial contamination. The cell cultures have tested negative for HIV 1 & 2, Hepatitis A, B, & C, HTLV, *Mycoplasma spp*., other bacteria and fungi.

Contents and Storage
One vial of 1 x 10^{6} cells in cryopreservation media (CryoStor® CS5, Sigma Aldrich).

Handling Procedures

Biosafety Level 1 safety procedures are recommended when handling this cell line.

Required Materials

Reagent	Recommended Supplier	Part Number
Defined Keratinocyte-SFM kit (with growth supplement)	Gibco	10744-019
Fetal Bovine Serum (Qualified)	Gibco	26140-079
TrypLE™ Express (*or trypsin*)	Gibco	12605-010

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Materials
Cell Culture Treated Flask, T-25
Cell Culture Treated Flask, T-75
70% Ethanol (EtOH)
Sterile Conical Tubes, 50ml
Phosphate Buffered Saline (PBS) or equivalent
Freezing Medium of Choice (CryoStor CS5)

Equipment
Biological Safety Cabinet (BSC) - Level I
Centrifuge
Incubator: 37°C, 5% CO2
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: Prostate Cell Lines Culture Medium

Component	Stock Concentrations	Final Concentrations	Amount added to 500ml
Defined Keratinocyte-SFM	-	-	489ml
EGF Growth supplement* (vial supplied with media kit) *Insulin, EGF, fibroblast growth factor (FGF)	-	-	1ml
Fetal Bovine Serum (Qualified), **heat-inactivated**	-	2%	10ml

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. After retrieval of cell vial from cryo-storage, 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.

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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:

Table 2: Cell seeding volume

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

Note: *Cells are density dependent. At Cell Thaw, entire contents of vial should be used to seed one T-25 flask*.

4. Gently rock the culture flasks to evenly distribute the cells.
5. Place flask into a 37°C incubator at 5% CO_2.
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: *The cells typically reach maximum confluence in approximately 3 days (OPCT lines) or 5 days (OPCN lines) +/- 1-2 days depending on split ratio.*
   a. If cells are less than 90% confluent, perform steps 3-5.
   b. If cells are at least 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% CO_2.
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 (PBS) 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.

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a. Passage cells every 3-7 days at 1:3 to 1:6 split ratio.
b. Refer to Table 2 for cell seeding volumes.

Freezing Cells

1. Place a controlled rate freezing unit (i.e. 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 controlled rate freezing unit to liquid nitrogen vapor phase or -80°C storage.

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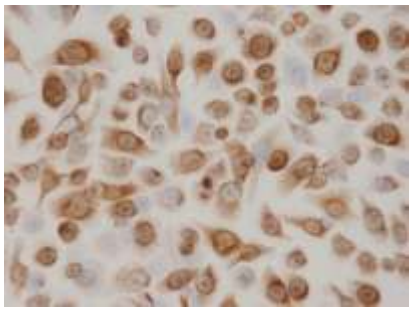
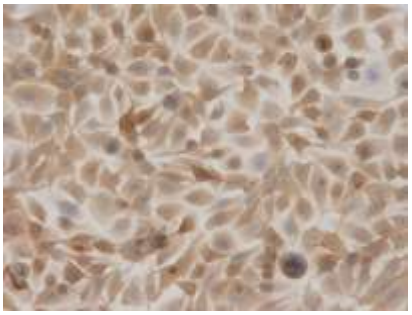
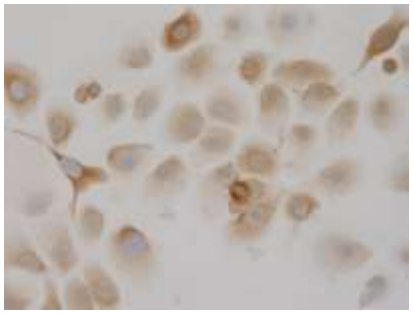
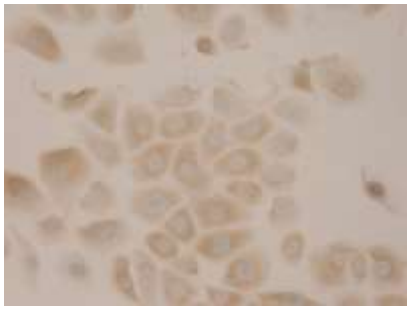
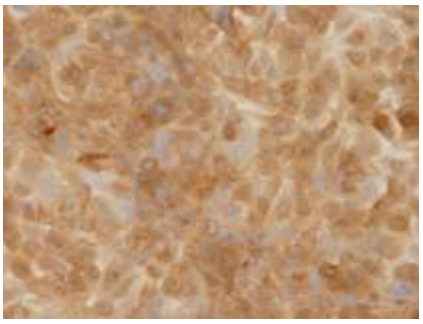
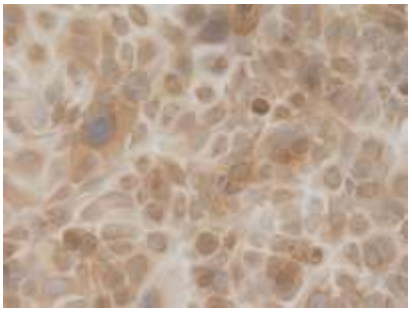
APPENDIX A: Cell Culture Images

Normal cell lines	Tumor cell lines
	
Picture 1: OPCN-1 cells in culture	**Picture 2: OPCT-1 cells in culture**
	
Picture 3: OPCN-2 cells in culture	**Picture 4: OPCT-2 cells in culture**
	
Picture 5: OPCN-3 cells in culture	**Picture 6: OPCT-3 cells in culture**

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