

Data Sheet

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BCRJ Code:	0389
Cell Line:	RWPE-1
Species:	Homo sapiens
Vulgar Name:	Human
Tissue:	Prostate
Cell Type:	Epithelial
Morphology:	Epithelial
Disease:	Normal
Growth Properties:	Adherent
Sex:	Male
Age/Ethnicity:	54 Year /
Derivation:	Epithelial cells derived from the peripheral zone of a histologically normal adult human prostate were transfected with a single copy of the human papilloma virus 18 (HPV-18).
Tumor Formation::	No
Products:	Antigen Expression kallikrein 3, KLK3 (prostate specific antigen, PSA); Homo sapiens, expressed (upon exposure to androgen) Receptor Expression: androgen receptor, expressed Genes Expressed: cytokeratin 18, cytokeratin 8 Tumor Suppressor Gene(s): p53 +, pRB Cellular Products: cytokeratin 18; cytokeratin 8
Biosafety:	2

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Culture Medium:

The base medium for this cell line is provided by Invitrogen (GIBCO) as part of a kit: Keratinocyte Serum Free Medium (K-SFM), Kit Catalog Number 17005-042. This kit is supplied with each of the two additives required to grow this cell line (bovine pituitary extract (BPE) and human recombinant epidermal growth factor (EGF). To make the complete growth medium, you will need to add the following components to the base medium: - 0.05 mg/ml BPE - provided with the K-SFM kit - 5 ng/ml EGF - provided with the K-SFM kit. NOTE: Do not filter complete medium.

Subculturing:

Volumes are given for a 75 cm² flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes. Remove and discard culture medium. Briefly rinse the cell layer with Ca⁺⁺/Mg⁺⁺ free Dulbecco's phosphate-buffered saline (D-PBS). Add 2.0 to 3.0 mL (to a T-25 flask) or 3.0 to 4.0 mL (to a T-75 flask) of 0.05% Trypsin - 0.53mM EDTA solution, diluted 1:1 with D-PBS, and place flask in a 37°C incubator for 5 to 8 minutes. Observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 10 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Add 6.0 to 8.0 mL of 0.1% Soybean Trypsin Inhibitor (ATCC® 30-2014™) or 2% fetal bovine serum in D-PBS, as appropriate, and aspirate cells by gently pipetting. Transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 7 minutes. Discard supernatant and resuspend cells in fresh serum-free growth medium. Add appropriate aliquots of cell suspension to new culture vessels. An inoculum of 2 X 10⁴ to 4 X 10⁴ viable cells/cm² is recommended. Incubate cultures at 37°C. We recommend that you maintain cultures at a cell concentration between 4 X 10⁴ and 7 X 10⁴ cells/cm². Cells grown under serum-free or reduced serum conditions may not attach strongly during the 24 hours after subculture and should be disturbed as little as possible during that period. NOTE: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010.

Subculturing Medium Renewal:

Every 2 days

Subculturing Subcultivation Ratio:

1:3 to 1:5

Culture Conditions:

Atmosphere: air, 95%; carbon dioxide (CO₂), 5% Temperature: 37°C

Cryopreservation:

95% FBS + 5% DMSO (Dimethyl sulfoxide)

Thawing Frozen Cells:

SAFETY PRECAUTION: It is strongly recommended to always wear protective gloves, clothing, and a full-face mask when handling frozen vials. Some vials may leak when submerged in liquid nitrogen, allowing nitrogen to slowly enter the vial. Upon thawing, the conversion of liquid nitrogen back to its gas phase may cause the vial to explode or eject its cap with significant force, creating flying debris.

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as its contents are thawed and decontaminate it by dipping in or spraying with 70% ethanol. From this point, all operations must be performed under strict aseptic conditions.
3. For cells sensitive to DMSO, it is recommended to remove the cryoprotective agent immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at approximately $125 \times g$ for 5 to 7 minutes.
4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (see specific batch information for the appropriate dilution ratio).
5. Incubate the culture under appropriate atmospheric and temperature conditions (see "Culture Conditions" for this cell line).

NOTE: It is important to avoid excessive alkalinity of the medium during cell recovery. To minimize this risk, it is recommended to place the culture vessel containing the growth medium in the incubator for at least 15 minutes before adding the vial contents. This allows the medium to stabilize at its normal pH (7.0 to 7.6).

References:

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