Data Sheet

Cell Line:	Human keratinocyte (hKT)
BBRJ Code:	nh-skp-KT0015
Product Type:	Primary cells
Species:	Homo sapiens
Vulgar Name:	Human
Tissue:	Skin, Foreskin
Cell Type:	Keratinocyte
Morphology:	Polygonal
Growth Properties:	Adherent
Sex:	Male
Age/Ethinicity:	4 Year / Black
Derivation:	Established from human foreskin
Applications:	In vitro Assays for Research and Industry
Biosafety:	2
Culture Medium:	Keratinocyte Basal Medium (KBM) supplemented with Keratinocyte Growth Medium (KGM)-Lonza

Subculturing:	Enzymatic Dissociation: 1. Remove and discard the culture medium. 2. Rinse the flask three times with 1x PBS solution to remove residual metabolites from cellular metabolism. 3. Add 2.0 to 3.0 mL of trypsin-EDTA solution to the flask. 4. Observe the flask under an inverted microscope until the cell layer becomes individualized and detached (usually between 3 and 7 minutes). NOTE: To prevent cell clumping, do not agitate the flask until trypsin has effectively acted. The flask may be placed at 37ºC (optimum trypsin activity temperature) to optimize the process. If, within the expected time, the cells are individualized but still slightly adherent, the flask can be gently tapped against the palm of the hand or a flat, smooth surface. 5. Add a volume of complete growth medium proportional to the previously added trypsin solution (2.0–3.0 mL). 6. Gently mix the cell suspension with the pipette to ensure even distribution of trypsin and medium, then transfer the suspension to a tube. 7. Remove an aliquot for cell counting using a Neubauer chamber or an automated cell counter. 8. Centrifuge the cell suspension. 9. Subculture: Cultures can be established by centrifugation followed by resuspension at a density of 4–6 × 10^3 cells/cm^2. NOTE: For more details on enzymatic dissociation and cell subculture, refer to Chapter 12 of Culture of Animal Cells, 6th edition, by R. Ian Freshney, published by Alan R. Liss, N.Y., 2010.
Subculturing Medium Renewal:	Every 2 to 3 days
Cryopreservation:	50% FBS +40% KBM + 10% 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 × 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:

R. Ian Freshney's book Culture of Animal Cells, 6th edition, published by Alan R. Liss, N.Y., 2010.