Data Sheet

Cell Line:	Human keratinocyte (hKT)
BBRJ Code:	nh-skp-KT0109
Product Type:	Primary cells
Species:	Homo sapiens
Vulgar Name:	Human
Tissue:	Foreskin
Cell Type:	Keratinocyte
Morphology:	Polygonal
Growth Properties:	Adherent
Sex:	Male
Age/Ethinicity:	13 years / Brown
Phototype:	4
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. 3. Add 2.0–3.0 mL of 0.05% 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 trypsin inhibitor proportional to the volume of trypsin added (2.0–3.0 mL). 6. Gently pipette the suspension to ensure uniform cell distribution and enzyme neutralization, then transfer to a centrifuge tube. 7. Remove an aliquot for cell counting. 8. Centrifuge the cell suspension at 150 × g for 5 minutes. 9. Subculture: After centrifugation, resuspend the cells in growth medium and seed at a density of 4–6 × 10^3 cells/cm^2. NOTE: For further information on enzymatic dissociation and cell subculture, refer to Chapter 12 of Culture of Animal Cells, 6th edition, by R. Ian Freshney (Alan R. Liss, New York, 2010).
Subculturing Medium Renewal:	Every 2 to 3 days
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO2), 5% Temperature: 37°C
Cryopreservation:	50% FBS +40% KBM + 10% DMSO (Dimethyl sulfoxide)

Thawing Frozen Cells:	SAFETY PRECAUTIONS: It is strongly recommended to use gloves, protective clothing, face shields, and safety glasses when handling frozen cryotubes. It is important to note that some cryotubes may leak when submerged in liquid nitrogen and can slowly fill with nitrogen. During the thawing process, the conversion of liquid nitrogen into its gaseous form may cause cryotubes and their lids to explode. Step-by-Step Thawing: 1. Thaw the cryotube by gently shaking it in a water bath at 37°C. To minimize contamination, keep the cryotube cap out of the water. Thawing should be rapid (approximately 2 minutes). 2. Immediately after the entire contents of the cryotube have thawed, remove the vial from the water bath. Decontaminate the vial by either dipping it in or spraying it with 70% ethanol. NOTE: All procedures must be carried out under strict aseptic conditions. 3. Transfer the contents of the cryotube to a sterile 15 mL centrifuge tube containing 9.0 mL of culture medium. Gently pipette the suspension to ensure homogenization. Do not centrifuge. 4. Transfer the cell suspension to the appropriate number of culture flasks (refer to batch-specific information for the recommended culture dilution ratio). 5. Incubate the culture in an atmosphere of 5% CO_2 at 37°C. NOTE: To prevent excessive alkalinity of the medium during cell recovery, it is recommended to place the culture flasks containing the appropriate medium for maintenance and cell growth in the incubator for at least 15 minutes before adding the thawed cells. This allows the culture medium to reach its normal pH range (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.