Data Sheet

Cell Line:	Human Fibroblast (hFB)
BBRJ Code:	nh-skp-FB0040
Product Type:	Primary cells
Species:	Homo sapiens
Vulgar Name:	Human
Tissue:	Foreskin
Cell Type:	Fibroblast
Morphology:	Spindle-shaped
Growth Properties:	Adherent
Sex:	Male
Age/Ethinicity:	2 years / Black
Derivation:	Established from human foreskin
Applications:	In vitro Assays for Research and Industry
Biosafety:	2
Culture Medium:	Dulbecco's Modified Eagle's Medium (DMEM) Low glucose with fetal bovine serum to a final concentration of 10%.

Subculturing:	Enzymatic Dissociation: 1. Remove and discard the culture medium. 2. Rinse the flask three times with 1x PBS solution to remove residual metabolites and serum traces. 3. Add 2.0 to 3.0 mL 0.125 % 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 medium proportional to the trypsin volume (2.0–3.0 mL) to inactivate the enzyme. 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 5-7 minutes. 9. Subculture: After centrifugation, resuspend the cells in fresh growth medium and seed at a density of 4 - 6 x 10^3 cells/cm^2. NOTE: For more information on enzymatic dissociation and cell subculture, see the 12th chapter of R. Ian Freshney's book Culture of Animal Cells, 6th edition, published by Alan R. Liss, N.Y., 2010.
Subculturing Medium Renewal:	Every 2 to 3 days
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO_2), 5% Temperature: 37°C
Cryopreservation:	50% FBS + 40% DMEM + 10% DMSO (Dimethyl sulfoxide)

Thawing Frozen Cells:	SAFETY PRECAUTIONS: It is strongly recommended that gloves, protective clothing, face shields, and safety glasses be worn when handling frozen cryotubes. Some cryotubes may leak when submerged in liquid nitrogen, allowing the tube to fill with liquid. During thawing, the rapid expansion of liquid nitrogen into gas can cause the cryotube or its lid 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 operations must be carried out under strict aseptic conditions. 3. For DMSO-sensitive cells, it is recommended to remove the cryoprotective agent immediately. Transfer the contents of the cryotube to a 15 mL sterile centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at 125 x g for 5 to 10 minutes. 4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (refer to batch-specific documentation for the recommended seeding ratio). 5. Incubate the culture at 37°C in a 5% CO2 atmosphere. 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.