Data Sheet

Cell Line:	Human Fibroblast (hFB)
BBRJ Code:	nh-skp-FB0048
Product Type:	Primary cells
Species:	Homo sapiens
Vulgar Name:	Human
Tissue:	Skin, Foreskin
Cell Type:	Fibroblast
Morphology:	Spindle Shaped
Growth Properties:	Adherent
Sex:	Male
Age/Ethinicity:	3 Year / White
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 bottle three times with 1x PBS solution to remove remnants from cellular metabolism. 3- Add 2.0 to 3.0 mL trypsin-EDTA solution to the flask. 4- Observe the bottle under the inverted microscope until the cell layer is individualized and derelict (usually between 3 and 7 minutes). NOTE: In order to avoid the breakdown of the cells into clusters, the bottle should not be stirred until the effective action of the trypsin. The bottle can be placed at 37ºC (optimum trypsin temperature) to optimize the process. If, during the expected time, the cells are individualized but still adhered, the bottle can be shaken moderately against the palm of the hands or flat and smooth surface. 5- Add complete growth medium in volume proportional to the previously placed trypsin solution (2.0-3.0 mL). 6- Shake with the pipette the cell solution with trypsin and medium and transfer gently to a tube. 7- Remove an aliquot for counting in Neubauer's chamber. 8- Centrifuge the cell suspension. 9- Subculture: Cultures can be established by centrifugation with subsequent resuspension at 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
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 glasses be used when handling frozen cryotubes. It is important to note that some cryotubes leak when submerged in liquid nitrogen and are slowly filled with nitrogen. During the process of defrost, the conversion of liquid nitrogen into its gas form may result in the explosion of cryotubes and their lids. Step-by-step thawing: 1. Thaw the cryotube by shaking gently in a water bath at 37 ° C. To reduce the contamination, keep cryotube cover out of the water. Thawing should be quick (approximately 2 minutes). 2. Remove the vial from the water bath immediately upon thawing of the contents of the interior of the cryotube. Perform the decontamination of this by dipping it or spraying it with 70% ethanol. Note: All operations must be carried out under strict aseptic conditions. 3. For cells sensitive to DMSO, it is recommended that the cryoprotective agent be removed immediately. Transfer the contents of the cryotube to a sterile centrifuge tube (15 mL) containing 9.0 mL of culture medium and centrifuge for approximately 125G for 5 to 10 minutes. 4. Discard the supernatant and resuspend the cell pellet with the recommended complete medium (see batch-specific information for recommended culture dilution ratio). 5. Incubate the culture in an atmosphere of 5% CO2 at 37ºC. NOTE: It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that the culture bottle with the medium suitable for maintenance and cell growth be allocated in the incubator prior to the addition of the thawed cells to the bottle for at least 15 minutes. In this way, the culture medium will reach 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.