Data Sheet

Cell Line:	Human Melanocyte (hML)
BBRJ Code:	nh-skp-ML0028
Product Type:	Primary cells
Species:	Homo sapiens
Vulgar Name:	Human
Tissue:	Skin, Foreskin
Cell Type:	Melanocyte
Morphology:	Bipolar, multipolar/dendritic
Growth Properties:	Adherent
Sex:	Male
Age/Ethinicity:	6 Year / Brown
Derivation:	Established from human foreskin
Applications:	In vitro Assays for Research and Industry
Biosafety:	2
Culture Medium:	MBM-4 Melanocyte Basal Medium 4 (MBM-4) supplemented with Melanocyte Growth Medium 4 (MGM-4) - Lonza.

Subculturing:	Enzymatic Dissociation: 1- Remove and discard the culture medium. 2- Rinse the bottle briefly with 1x PBS solution to remove remnants from cellular metabolism. 3- Add 2 to 3 mL trypLE Select enzyme solution to the flask. 4- Observe the bottle under the inverted microscope until the cell layer is individualized and derelict (usually between 3 and 10 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 trypLE. The bottle can be placed at 37º C 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 2 - 3 mL of complete medium to the flask. 6 - Tilt the flask in all directions to thoroughly rinse the flask. Transfer the cell suspension to a 15-mL conical tube. 7- Remove an aliquot for counting in Neubauer's chamber. 8. Centrifuge the cell suspension at 100× g for 5–10 minutes. 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% MBM-4 +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.