Code:	nh-skp-ML0028
Cell Line:	Human Melanocyte (hML)-nh-skp-ML0028
Species:	Homo sapiens **Vulgar Name:** Human
Tissue:	Foreskin
Morphology:	Bipolar/oval or fusiform, multipolar/ dendritic
Growth Properties:	Adherent
Age Ethinicy:	6 years / brown
Derivation:	Established from human foreskin
Applications:	In vitro Assays for Research and Industry
Biosafey:	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.

Medium Renewal: Every 2 to 3 days

Subcultivation ratio:

Culture Conditions:	Atmosphere: air 95% and carbon dioxide (CO_2) 5%
Cryopreservation:	50% FBS +40% MBM-4 +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% CO_2 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.