Protocol for cryopreservation of HBEC3-KT

Version: May 2021

Evercyte Ord. No.:	CkHT-004-0230
Designation:	HBEC3-KT, human bronchial epithelial progenitor cells
Freezing medium:	Growth medium for HBEC3-KT cells (Keratinocyte-SFM Kit, Gibco, see protocol *Passaging of HBEC3-KT cells*) 10 % FBS (Sigma-Aldrich, Cat# F7524) 10 % DMSO (Sigma-Aldrich, Cat# D2650) Preparation of 10 ml freezing medium, prepare just before use: - take 8 ml of growth medium and transfer to 15 ml centrifugation tube - add 1 ml of FBS - add 1 ml of DMSO - mix properly and store at 4°C
Additional reagents:	0.025 % Trypsin-EDTA (Gibco, Cat# R-001-100) Defined Trypsin Inhibitor (Gibco, Cat# R007100) PBS (Sigma-Aldrich, Cat# D8537) 0.1 % Gelatin from porcine skin – Type A (Sigma-Aldrich, Cat# G1890) 2 % FBS in PBS (FBS: Sigma-Aldrich, Cat# F7524)
Freezing cells:	- detach the cells from the culture vessel by using Trypsin-EDTA solution as described in protocol *Passaging of HBEC3-KT cells* - resuspend the detached cells in growth medium and centrifuge at 170 g for 5 min - discard the supernatant - resuspend the resulting cell pellet in the remaining droplet - add freezing medium (tempered to 4°C) to reach a cell density of about 1 x 10^6 cells/ml (for thawing in a 25 cm^2 culture flask) - add 1 ml of this cell suspension to each pre-cooled cryovial and immediately transfer the cells to -80°C - after 24 hours transfer the vials to the liquid nitrogen tank
Thawing cells:	**When you start cultivating the cells, please transfer the content of the original Evercyte vial containing HBEC3-KT cells into a T25 roux flask as described in the following:** - coat a 25 cm^2 culture flask with porcine Gelatin solution (Sigma-Aldrich, Cat# G1890; diluted to 0.1 % in PBS) as also described in protocol *Passaging of HBEC3-KT cells*: therefore, the culture flasks are treated with Gelatin solution (80 µl/cm^2) at 37°C for 4 hours (up to one week). Before introducing the cells, remove excess of Gelatin solution. Use the pre-coated flasks immediately for seeding of cells, the surface must not dry out.

EVERCYTE GmbH
Leberstrasse 20, 1110 Vienna, AUSTRIA
office@evercyte.com | www.evercyte.com

- add 6 ml of growth medium to a 25 cm² pre-coated culture flask and place it in the incubator for at least 30 min to allow the medium to reach 37°C and its normal pH
- take a vial of frozen cells, rinse it outside with ethanol and pre-warm in the hand until one last piece of frozen cells is seen
- then, immediately transfer the content of the vial to a 15 ml centrifugation tube pre-filled with 9 ml of medium pre-cooled to 4°C and centrifuge for 5 min at 170 g
- discard the supernatant and resuspend the cell pellet in the remaining droplet
- add 1 ml of the pre-warmed medium to the cells, transfer the cells to the prepared culture flask and incubate at 37°C in a suitable incubator
- perform a medium change 24 hours after thawing, if the cells are already 80-90 % confluent at this point, they have to be passaged as described in *Protocol passaging of HBEC3-KT cells*

EVERCYTE GmbH
Leberstrasse 20, 1110 Vienna, AUSTRIA
office@evercyte.com | www.evercyte.com