Protocol for passaging of HBEC3-KT

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Evercyte Ord. No.:	CkHT-004-0230
Designation:	HBEC3-KT, human bronchial epithelial progenitor cells
Growth medium:	Keratinocyte-SFM Kit (Gibco, Cat# 17005-042): Final components: Keratinocyte-SFM basal medium (Gibco, Cat# 17005042) Bovine Pituitary Extract (Gibco, Cat# 17005042) EGF (Gibco, Cat# 17005042) - take one bottle of Keratinocyte-SFM (1x) basal medium (500 ml) - add 25 mg of BPE (Keratinocyte-SFM component) - add 2.5 µg of EGF (Keratinocyte-SFM component) - mix properly, - store at 4°C for a maximum of 4 weeks - temper the medium to room temperature (not 37°C) before use
Coating:	0.1 % Gelatin solution from porcine skin – Type A The coating solution is prepared by mixing the following components: Powdered Gelatin from porcine skin – Type A (Sigma-Aldrich, Cat# G1890, stored at 4°C) Cell Culture Grade Water (HyClone, Cat# SH30529.03, ready-to-use, stored at RT) - weigh 2 g of Gelatin in glass bottle - add 200 ml Cell Culture Grade Water - transfer bottle to water bath to dissolve Gelatin - autoclave resulting 1 % Gelatin solution - aliquot 5 ml each - store at 4°C For coating of a T25 roux flask proceed as follows: - liquefy the Gelatin solution at 37°C - add 45 ml of Cell Culture Grade Water to 5 ml 1 % Gelatin solution and mix carefully - store resulting 0.1 % Gelatin solution at 37°C - transfer 2 ml of diluted Gelatin solution (0.1 %) to the T25 roux flask (80 µl/cm²) - completely wet the surface of the culture flask - incubate the culture flask at 37°C at least for 4 hours (up to one week)

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	- remove excess of Gelatin solution - use culture flask immediately for seeding of cells, the surface must not dry out
Additional reagents:	0.025 % Trypsin-EDTA (Gibco, Cat# R-001-100, ready-to-use, stored at 4°C) Defined Trypsin Inhibitor (Gibco, Cat# R007100, ready-to-use, stored at 4°C) PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at RT) 0.1 % Gelatin from porcine skin – Type A (Sigma-Aldrich, Cat# G1890) 2 % FCS in PBS (FBS: Sigma-Aldrich, Cat# F7524, stored at 4°C)
Passaging of cells:	- the new culture flasks have to be coated with Gelatin as described above - remove and discard the culture medium - wash the cells twice with PBS (each 160 µl/cm²), remove PBS completely - add Trypsin-EDTA solution (20 µl/cm²), make sure that all cells have been in contact with this solution - incubate the culture flask at 37°C for approximately 2-3 min - observe cell detachment under an inverted microscope - as soon as all cells are detached (if necessary, agitate the cells by gently hitting the flask) halt the Trypsin action by addition of 2 % FCS in PBS (220 µl/cm²) - centrifuge at 170 g for 5 min - discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium (about 160 µl/cm²) - transfer appropriate aliquots of the cell suspension to Gelatin-coated culture vessels supplemented with growth medium (final volume of 240 µl/cm²) - a split ratio of 1:4 twice a week is recommended (after having reached about 80-90 % confluence) - perform a medium change after 2-3 days if cells have not reached required cell density - cultivate cells at 37°C in a humidified atmosphere with 5% CO_2

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