Protocol for passaging of NHEK/SVTERT3-5

Version: February 2024

Evercyte Ord. No.:	CLHT-011-0026-5
Designation:	NHEK/SVTERT3-5, human epidermal keratinocytes
Growth medium:	KGM™ Gold Keratinocyte Growth Medium BulletKit™ (Lonza, Cat# 00192060) supplemented with G418: Final components: KBM™ Gold™ Basal Medium (Lonza, Cat# 00192151) Components of KGM™ Gold™ SingleQuots™ supplements (Lonza, Cat# 00192152: BPE, hEGF, Insulin, Hydrocortisone, Transferrin, Epinephrine, without GA) 50 µg/ml G418 (InvivoGen, Cat# ant-gn5, 100 mg/ml stock solution, ready-to-use) - take one bottle of KBM™ Gold™ Basal Medium (500 ml) - add 2 ml of BPE (component of KGM™ Gold™ SingleQuots™) - add 500 µl of hEGF (component of KGM™ Gold™ SingleQuots™) - add 500 µl of Insulin (component of KGM™ Gold™ SingleQuots™) - add 500 µl of Hydrocortisone (component of KGM™ Gold™ SingleQuots™) - add 500 µl of Transferrin (component of KGM™ Gold™ SingleQuots™) - add 250 µl of Epinephrine (component ofKGM™ Gold™ SingleQuots™) - add 250 µl of G418 stock solution (100 mg/ml) - mix properly - store at 4°C for a maximum of 4 weeks - temper the medium to room temperature (not 37°C) before use
Additional reagents:	0.05% Trypsin-EDTA (Gibco, Cat# 25300-054, 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)
Passaging of cells:	- remove and discard the culture medium - wash the cells once with PBS (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, cells have typically rounded up but have not detached yet - tap side of the flask to apply mechanical force and further incubate for 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), add Defined Trypsin Inhibitor (20 µl/cm²)

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- resuspend the cells in growth medium (about 160 µl/cm²) and aspirate the cells by pipetting
- pass cells through EASY strainer 100 µm (Greiner Cat# 542000)
- centrifuge at 170 g for 5 min
- discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium
- transfer appropriate aliquots of the cell suspension to new culture vessels supplemented with growth medium (final volume of 240 µl/cm²)
- a split ratio of 1:4 to 1:6 twice a week is recommended (after having reached about 60-70 % 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