# Protocol for passaging of NHEK/SVTERT3-5

Version: May 2021

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| Evercyte Ord. No.: | CLHT-011-0026-5 |
| Designation: | NHEK/SVTERT3-5, human epidermal keratinocytes |
| Growth medium: | KGM™-2 BulletKit™ (Lonza, Cat# CC-3107) supplemented with G418:<br><br>Final components:<br>KBM™-2 basal medium (Lonza, Cat# CC-3103)<br>Components of KGM™-2 SingleQuots (Lonza, Cat# CC-4152: BPE, hEGF, insulin, hydrocortisone, transferrin, epinephrine, w/o GA)<br>50 µg/ml G418 (InvivoGen, Cat# ant-gn5, 100 mg/ml stock solution, ready-to-use)<br><br>- take one bottle of KGM™-2 basal medium (500 ml)<br>- add 2 ml of BPE (component of KGM™-2 SingleQuots™)<br>- add 500 µl of hEGF (component of KGM™-2 SingleQuots™)<br>- add 500 µl of Insulin (component of KGM™-2 SingleQuots™)<br>- add 500 µl of Hydrocortisone (component of KGM™-2 SingleQuots™)<br>- add 500 µl of Transferrin (component of KGM™-2 SingleQuots™)<br>- add 500 µl of Epinephrine (component of KGM™-2 SingleQuots™)<br>- add 250 µl of G418 stock solution (100 mg/ml)<br><br>- mix properly<br>- store at 4°C for a maximum of 4 weeks<br>- 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)<br>Defined Trypsin Inhibitor (Gibco, Cat# R007100, ready-to-use, stored at 4°C)<br>PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at RT) |
| Passaging of cells: | - remove and discard the culture medium<br>- wash the cells once with PBS (160 µl/cm²), remove PBS completely<br>- add Trypsin-EDTA solution (20 µl/cm²), make sure that all cells have been in contact with this solution<br>- incubate the culture flask at 37°C for approximately 4-5 min<br>- observe cell detachment under an inverted microscope<br>- as soon as all cells are detached (if necessary, agitate the cells by gently hitting the flask), add Defined Trypsin Inhibitor (20 µl/cm²)<br>- resuspend the cells in growth medium (about 160 µl/cm²) and aspirate the cells by pipetting<br>- centrifuge at 170 g for 5 min |

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- 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 1:2 to 1:3 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% $CO_2$

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