

Protocol for passaging of LF/TERT309

Version: January 2022

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| Evercyte Ord. No.: | CHT-067-0309 |
| Designation: | LF/TERT309, human lung fibroblasts |
| Growth medium: | DMEM/F12 (1:1), w: stable Glutamine, w: 1.2 g/L NaHCO ₃ (PAN-Biotech) supplemented with FBS and G418 |
| | <p><u>Final components:</u></p> <p>DMEM/F12 (1:1), w: stable Glutamine, w: 1.2 g/L NaHCO₃ (PAN-Biotech Cat# P04-41150)</p> <p>10 % FBS (PAN-Biotech, Cat# P30-3031, ready-to-use, stored at 4°C after thawing)</p> <p>200 µg/ml G418 (InvivoGen, Cat# ant-gn5, 100 mg/ml, ready-to-use, stored at -20°C)</p> <ul style="list-style-type: none"> - take one bottle of DMEM/F12 (1:1) basal medium (500 ml) and remove 51 ml - add 50 ml of fetal bovine serum (ready-to-use) - add 1 ml of G418 stock solution (100 mg/ml, ready-to-use) - mix properly and 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 after thawing) PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at room temperature) |
| Passaging of cells: | <ul style="list-style-type: none"> - remove and discard the culture medium - wash the cells once with PBS (each 160 µl/cm²), remove PBS completely - add Trypsin-EDTA solution (20 µl/cm²), wet surface completely - incubate the culture flask at 37°C for approximately 2-3 min for complete cell detachment - observe cell detachment under an inverted microscope - as soon as all cells are detached (if necessary, shake the flask for complete cell detachment) - add growth medium (about 160 µl/cm²) and aspirate the cells by pipetting - 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 1:4-1:6 twice a week is recommended (after having reached about 70-90 % confluence) - cultivate cells at 37°C in a humidified atmosphere with 5% CO₂ |
| Related products: | HDF/TERT164, dermal fibroblasts, adult donor (Evercyte, Cat# CHT-008-0164) fHDF/TERT166, foreskin fibroblasts (Evercyte, Cat# CHT-031-0166) |