Protocol for passaging of HDF/TERT164

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Evercyte Ord. No.:	CHT-008-0164
Designation:	HDF/TERT164, human fibroblasts
Growth medium:	DMEM/Ham´s F12 supplemented with FBS and G418: <u>Final components:</u> DMEM/Ham´s F12 (1:1) (PAN Biotech, Cat# P04-41150) 10 % FBS (PAN-Biotech, Cat# P30-3031) 100 µg/ml G418 (InvivoGen, Cat# ant-gn-5, 100 mg/ml stock solution, ready-to-use) - take one bottle of DMEM/Ham´s F12 basal medium (500 ml) and remove 50.5 ml - add 50 ml FBS - add 500 µl G418 stock solution - 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:	PBS (Sigma-Aldrich, Cat# D8537) 0.05 % Trypsin-EDTA (Gibco, Cat#25300-054, ready-to-use, stored at 4°C after thawing)
Passaging of cells:	- remove and discard the culture medium - wash the cells once with PBS (160 µl/cm²), remove PBS completely - add 0.05 % Trypsin-EDTA solution (20 µl/cm^2), make sure that all cells have been in contact with this solution - incubate the culture flask at 37°C for approximately 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 growth medium (about 160 µl/cm²) and aspirate the cells by pipetting - add appropriate aliquots of the cell suspension to new culture vessels supplemented with growth medium (final volume of 240 µl/cm²). - transfer appropriate aliquots of the cell suspension to 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 90 % confluence) - cultivate cells at 37°C in a humidified atmosphere with 5% CO_2
Related products:	HDF/TERT164, human fibroblasts, adult (Evercyte, Cat# CHT-008-0164) fHDF/TERT166, human fibroblasts, foreskin (Evercyte, Cat# CHT-031-0166)

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