

## Protocol for passaging of fHDF/TERT166

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Evercyte Ord. No.:	CHT-031-0166
Designation:	fHDF/TERT166, human foreskin fibroblasts
Growth medium:	<p>DMEM/F12 (1:1), w: stable Glutamine, w: 1.2 g/L NaHCO<sub>3</sub> (PAN-Biotech) supplemented with FBS and G418:</p> <p><u>Final components:</u>                      DMEM/F12 (1:1), w: stable Glutamine, w: 1.2 g/L NaHCO<sub>3</sub> (PAN-Biotech Cat# P04-41150)                      10 % FBS (Sigma-Aldrich, Cat# F7524, ready-to-use, stored at 4°C after thawing)                      100 µg/ml G418 (InvivoGen, Cat# ant-gn5, 100 mg/ml, ready-to-use, stored at -20°C)</p> <ul style="list-style-type: none"> <li>- take one bottle of DMEM/F12 (1:1) basal medium (500 ml) and remove 50.5 ml</li> <li>- add 50 ml of fetal bovine serum (ready-to-use)</li> <li>- add 500 µl of G418 stock solution (100 mg/ml, ready-to-use)</li> <li>- mix properly and store at 4°C for a maximum of 4 weeks</li> <li>- temper the medium to room temperature (not 37°C) before use</li> </ul>
Additional reagents:	<p>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)</p>
Passaging of cells:	<ul style="list-style-type: none"> <li>- remove and discard the culture medium</li> <li>- wash the cells twice with PBS (each 160 µl/cm<sup>2</sup>), remove PBS completely</li> <li>- add Trypsin-EDTA solution (20 µl/cm<sup>2</sup>), wet surface completely</li> <li>- incubate the culture flask at 37°C for approximately 2-3 min for complete cell detachment</li> <li>- observe cell detachment under an inverted microscope</li> <li>- as soon as all cells are detached (if necessary, shake the flask for complete cell detachment)</li> <li>- add growth medium (about 160 µl/cm<sup>2</sup>) and aspirate the cells by pipetting</li> <li>- transfer appropriate aliquots of the cell suspension to new culture vessels supplemented with growth medium (final volume of 240 µl/cm<sup>2</sup>)</li> <li>- a split ratio of 1:4 twice a week is recommended (after having reached about 90 %)</li> <li>- cultivate cells at 37°C in a humidified atmosphere with 5% CO<sub>2</sub></li> </ul>
Related products:	HDF/TERT164, dermal fibroblasts, adult donor (Evercyte, Cat# CHT-008-0164)