Protocol for passaging of ASC/TERT1

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Evercyte Ord. No.:	CHT-001-0005
Designation:	ASC/TERT1, human mesenchymal stromal cells
Growth medium:	EGM™-2 Endothelial Cell Growth Medium-2 (Lonza, Cat# CC-3162) supplemented with FBS and G418: <u>Final components:</u> EBM™-2 basal medium (Lonza, Cat# CC-3156) Components of EGM™-2 SingleQuots™ (Lonza, Cat# CC-4176: Hydrocortisone, hFGF, VEGF, R3-IGF-1, Ascorbic acid, hEGF, Heparin) 2 % FBS (PAN Biotech, Cat# P30-3031) 200 µg/ml G418 (InvivoGen, Cat# ant-gn5, 100 mg/ml, ready-to-use) - take one bottle of EBM-2 basal medium (500 ml) - add 10 ml of FBS (ready-to-use) - add 200 µl of Hydrocortisone (EGM™-2 SingleQuots™) - add 2 ml of hFGF (EGM™-2 SingleQuots™) - add 500 µl VEGF (EGM™-2 SingleQuots™) - add 500 µl of R3-IGF-1 (EGM™-2 SingleQuots™) - add 500 µl of hEGF (EGM™-2 SingleQuots™) - add 500 µl of Heparin (EGM™-2 SingleQuots™) - add 500 µl of Ascorbic Acid (EGM™-2 SingleQuots™) - add 1 ml of 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:	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 twice with PBS (each 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 - 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)
- resuspend the cells in growth medium (about 160 µl/cm²) and aspirate the cells by pipetting
- 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 culture vessels supplemented with growth medium (final volume of 240 µl/cm²)
- a split ratio of 1:3 to 1:4 twice a week is recommended (after having reached about 80 % confluence)
- cultivate cells at 37°C in a humidified atmosphere with 5% CO_2

Related products:	WJ-MSC/TERT273, human mesenchymal stromal cells (Evercyte, Cat# CHT-021-0273)