Protocol for passaging of hTEC/SVTERT24-B

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Evercyte Ord. No.:	CLHT-010-0024-B
Designation:	hTEC/SVTERT24-B, human thymic epithelial cells
Growth medium:	OptiPRO™ SFM (Gibco) supplemented with GlutaMAX™-I and G418: Final components: OptiPRO™ SFM (Gibco, Cat# 12309-019) 2 mM GlutaMAX™-I (Gibco, Cat# 35050-038, ready-to-use, stored at RT) 50 µg/ml G418 (InvivoGen, Cat# ant-gn5, 100 mg/ml stock solution, ready-to-use, stored at -20°C) - take one bottle of OptiPRO™ SFM medium (500 ml) and discard 5 ml - add 5 ml of GlutaMAX™-I to the remaining 495 ml OptiPRO™ SFM medium - add 250 µl of G418 stock solution - mix properly - store at 4°C for a maximum of 4 weeks - temper the medium to RT (not 37°C) before use
Coating solution:	50 µg/ml Collagen I solution The coating solution is prepared by mixing the following components: Collagen I (Sigma-Aldrich, Cat# C2249, 3 mg/ml, stored at 4°C) PBS (Sigma-Aldrich, Cat# D8537, stored at RT) – take 29.5 ml PBS and transfer to a 50 ml centrifugation tube – add 0.5 ml of Collagen I solution (3 mg/ml stock solution) – mix carefully For coating of a T25 cell culture flask proceed as follows: – pipette 2 ml of diluted Collagen I solution (50 µg/ml) to a T25 roux flask – completely wet the surface of the culture flask (80 µl/cm^2) – incubate the culture flask for a minimum of 30 min at 37°C – remove excess of Collagen I solution – rinse culture flask once with PBS (160 µl/cm2) – use culture flask immediately for seeding of cells, the surface must not dry out
Additional reagents:	0.05 % Trypsin-EDTA (Gibco, Cat# 25300-054, stored at 4°C after thawing) Defined Trypsin Inhibitor (Gibco, Cat# R007100, stored at 4°C after thawing)

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

	PBS (Sigma-Aldrich, Cat# D8537, stored at RT) Collagen I solution (Sigma-Aldrich, Cat# C2249, 3 mg/ml stock solution, stored at 4°C, prepared see above)
Passaging of cells:	- 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), make sure that all cells have been in contact with this solution - incubate the culture flask at 37°C for approximately 1-3 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 Collagen I pre-coated culture vessels supplemented with growth medium (final volume of 240 µl/cm²) - a split ratio of 1:6 to 1:8 twice a week is recommended (after having reached about 90 %) - cultivate cells at 37°C in a humidified atmosphere with 5 % CO_2