Protocol for passaging of P-EP/SVTERT344

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Evercyte Ord. No.:	CLHT-053-0344
Designation:	P-EP/SVTERT344, human amniotic epithelial cells (hAEC), placental region
Growth medium:	EpiLife + S7 Supplement / DMEM + Glutamax + FBS (1:1) supplemented with G418

Final components:
49.5 % EpiLife (Gibco, Cat# MEPI500CA)
0.5 % S7 Supplement (Gibco, Cat# S0175)
40 % DMEM (1x) + Glutamax (Gibco, Cat# 61965-026)
10 % FBS (PAN Biotech, Cat# P30-3031, 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)

For preparation of 200 ml medium mix the following components:
- take 99 ml EpiLife
- add 1 ml S7 Supplement
- add 80 ml DMEM (1x) + Glutamax
- add 20 ml FBS
- add 200 µl 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

Coating: CellAdhere™ Type I Collagen, human

The coating solution is prepared by mixing the following components:
CellAdhere™ Type I Collagen, human (StemCell Technologies, Cat# 07005, 3 mg/ml)
0.01 N HCl (sterile filtered)

For preparation of 150 ml 0.01 N HCl mix the following components:
- take 15 ml HyPure Cell Culture Grade Water (HyClone, Cat# SH30529)
- add 200 µl 25 % HCl, Emsure (Merck, Cat# 1.00316.1011)
- add 135 ml HyPure Water to get 0.01 N HCl
- sterile filter with 0.22 µm filter

For coating of a T25 roux flask proceed as follows:
- transfer 2 ml 0.01 N HCl (80 µl/cm²) to a sterile tube
- add 10 µl Collagen I (1:200 dilution)
- transfer the coating solution to a T25 roux flask

	- completely wet the surface of the culture flask - incubate at room temperature for at least 1 hour - remove excess of coating solution - wash the flask once with PBS (160 µl/cm²) - remove PBS completely - use culture flask immediately for seeding of cells, the surface must not dry out
Additional reagents:	0.05% Trypsin-EDTA (Gibco, Cat# 25300-054, ready-to-use, stored at 4°C) PBS (Gibco, Cat# 14190-144, ready-to-use, stored at RT)
Passaging of cells:	- remove and discard the culture medium - wash the cells once with PBS (160 µl/cm²), remove PBS completely - add Trypsin-EDTA solution (20 µl/cm²), make sure that all cells have been in contact with Trypsin-EDTA and incubate the culture flask at 37°C for approximately 5-7 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) - add 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 1:6-1:8 twice a week is recommended (after having reached about 80% confluence) - cultivate cells at 37°C in a humidified atmosphere with 5 % CO_2

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