Protocol for passaging of hTCEpi

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Evercyte Ord. No.:	CHT-045-0237
Designation:	hTCEpi, human corneal epithelial cells
Growth medium:	KGM™-2 BulletKit™ (Lonza, Cat# CC-3107): Final components: KBM™-2 Basal Medium (Lonza, Cat# CC-3103) KGM™-2 SingleQuots™ Supplements (Lonza, Cat# CC-4152) - take one bottle of KBM™-2 Basal Medium (500 ml) - add 2 ml of BPE (KGM™-2 SingleQuots™ Supplements) - add 500 µl of hEGF (KGM™-2 SingleQuots™ Supplements) - add 500 µl of insulin (KGM™-2 SingleQuots™ Supplements) - add 500 µl hydrocortisone (KGM™-2 SingleQuots™ Supplements) - add 500 µl of transferrin (KGM™-2 SingleQuots™ Supplements) - add 500 µl of epinephrine (KGM™-2 SingleQuots™ Supplements) - GA-1000 (KGM™-2 SingleQuots™ Supplements) is not used - 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 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 4-5 min for complete detachment - observe cell detachment under an inverted microscope - as soon as all cells are detached (if necessary, shake 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

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- 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 1:8 twice a week is recommended (after having reached about 60-70 % confluence; never allow the culture to become completely confluent!)
- cultivate cells at 37°C in a humidified atmosphere with 5% CO_2

Related products:

HCEC-1CT, colonic epithelial progenitor cells (Evercyte, Cat# CkHT-039-0229)
PODO/TERT256, kidney tissue-derived podocytes (Evercyte, Cat# CHT-033-0256)
PODO/SVTERT152, urine-derived podocytes (Evercyte, Cat# CLHT-033-0152)

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