Protocol for passaging of HME1

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Designation:	HME1, human mammary epithelial cells
Growth medium:	MEGM™ BulletKit™ (Lonza, Cat# CC-3150) Final components: MEBM™ basal medium (Lonza, Cat# CC-3151) Components of MEGM™ SingleQuots™ (Lonza, Cat# CC-4136: BPE, hEGF, Insulin, Hydrocortisone) - take one bottle of MEBM™ basal medium (500 ml) - add 2 ml of BPE (MEGM™ SingleQuots™) - add 500 µl of hEGF (MEGM™ SingleQuots™) - add 500 µl of Insulin (MEGM™ SingleQuots™) - add 500 µl Hydrocortisone (MEGM™ SingleQuots™) - 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 3 min - observe cell detachment under an inverted microscope - as soon as all cells are detached add Defined Trypsin Inhibitor (20 µl/cm²), do not agitate the cells by hitting the flask - 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 new culture vessels supplemented with growth medium (final volume of 240 µl/cm²)

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- a split ratio of 1:3 twice a week is recommended (after having reached about 80-90%)
- cultivate cells at 37°C in a humidified atmosphere with 5% CO_2

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