Product-Data-Sheet for HME1

Version: May 2021

Evercyte Ord. No.:	CHT-044-0236
Designation:	HME1
Biosafety Level:	1
Shipped:	Frozen on dry ice
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)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Epithelial
Source:	Breast tissue (healthy female donor, 53 years)
Cell Type:	Human mammary epithelial cells
Antigen Expression:	Positive for Mucin-1, KRT8/18
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki
Comments:	HME1 cell line was developed from human mammary epithelial cells by ectopic expression of the catalytic subunit of human telomerase (hTERT). Growing cells show expression of the markers mucin-1 and luminal specific cytokeratins KRT8/18. Cells readily recover from cryopreservation as shown by longevity studies performed post thawing. No changes of the growth characteristics have been observed after thawing. The cell line was continuously cultured for more than 120 population doublings without showing signs of replicative senescence.
Propagation:	Cells are grown in MEGM™ BulletKit™ (see above) at 37°C in a humidified atmosphere with 5 % CO_2.
Subculturing:	For detachment of the cells remove and discard the culture medium and wash the cells once with PBS (about 160 µl/cm²). Remove PBS completely. Then, add 0.05 % Trypsin-EDTA solution (20 µl/cm²), make sure that all cells have been in contact with this solution

	and 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! Thereafter, 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. Then, add appropriate aliquots of the cell suspension to new culture vessels supplemented with growth medium (final volume of 240 µl/cm²). A split ratio of 1:3 twice a week is recommended (after cells have reached about 80-90 % confluence). Never allow the culture to become confluent!
Preservation:	Freezing medium: Cryostor® cell cryopreservation medium CS10 (Sigma-Aldrich, Ca# C2874, ready-to-use, stored at 4°C) Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach the cells from the culture vessel by using Trypsin-EDTA and Defined Trypsin Inhibitor (protocol *Passaging of HME1 cells*). Resuspend the detached cells in growth medium and centrifuge at 170 g for 5 min. Discard the supernatant, resuspend the resulting cell pellet in the remaining droplet and add freezing medium (tempered to 4°C) to reach a cell density of about 0.8-1.4 x 106 cells/ml (for thawing in a 25 cm² culture flask). Add 1 ml of this cell suspension to each pre-cooled cryovial and immediately transfer the cells to -80°C. After 24 hours transfer the vials to the liquid nitrogen tank Thawing of cells: **When you start cultivating the cells, please transfer the content of the original Evercyte vial containing HME1 cells into a T25 roux flask as described in the following:** Add 6 ml of growth medium to a 25 cm² culture flask and place the culture flask in the incubator for at least 30 min to allow the medium to reach 37°C and its normal pH. Take a vial of frozen cells, rinse it outside with ethanol and pre-warm in the hand until one last piece of frozen cells is seen. Then, immediately transfer the content of the vial to a 15 ml centrifugation tube pre-filled with 9 ml of medium pre-cooled to 4°C and centrifuge for 5 min at 170 g. Then, discard the supernatant and resuspend the cell pellet in the remaining droplet. Add 1 ml of the pre-warmed medium to the cells, transfer them to the prepared culture flask and incubate at 37°C in a suitable incubator. Perform a medium change 24 hours after thawing. If the cells are already 80 % confluent at this point, they have to be passaged (see above or protocol *Passaging of HME1* cells).
Doubling Time:	48 – 64 hours

Virus Testing	Cells have been tested negative for HAV and Parvo B19 with Roche DPX-PCR (cobas® TaqScreen DPX-Test), for HBV, HCV, HIV nucleic acids with Roche-Multiplex-PCR (cobas® TaqScreen MPX Test, v2.0).
Other Analytical Data:	Cells are negative for Mycoplasma contaminations as tested using MycoAlert™ Mycoplasma Detection Kit from Lonza. Cells are negative for bacterial and fungal contaminations as tested according to Ph. Eur. 2.6.1. / USP <71>. STR profile has been analyzed and is as expected.

Please Note:

The classification of biosafety level is based on Austrian Legislation (Gentechnikbuch; Systemverordnung) and on recommendations of the Central Committee on Biological Safety (ZKBS). While Evercyte undertakes all reasonable measures to test for absence of a selected panel of known human pathogenic viruses, there is currently no test procedure available that guarantees for complete absence of infectious pathogens. The use of state-of-the art infectious virus assays or viral antigen assays may leave open the possible existence of a latent viral genome, even if a negative test result is obtained. Therefore, we recommend that all human cell lines should be handled with caution such as an organism of ACDP Hazard Group 2.
People who work with our cells must follow national regulations and safety precautions. The laboratories must be equipped with a security level according to the classification of the cells / products. Evercyte assumes no liability whatsoever in connection with the receipt, handling or the consequences of improper use of our products.

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