EVERCYTE GmbH
the pharmacocellomics™ company
Muthgasse 18
1190 Wien
office@evercyte.com
www.evercyte.com

Product-Data-Sheet for HME1

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For questions please contact *office@evercyte.com*

Evercyte Ord. No.:	CHT-044-0236
Designation:	HME1
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	MEGM Bullet Kit (Lonza, Cat# CC-3150)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Epithelial-like
Source:	Human breast tissue (healthy donor)
Cell Type:	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). The cell line was continuously cultured for more than 120 population doublings without showing signs of replicative senescence[1]. 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

[1] Herbert B.-S., Wright W. E., Shay J. W. (2002) Oncogene, $p16^{INK4a}$ inactivation is not required to immortalize human mammary epithelial cells.

	thawing. No changes of the growth characteristics have been observed after thawing.
Propagation:	Cells are grown in MEGM medium (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. Remove PBS completely. Then, add 0.05 % Trypsin-EDTA (1x) solution (room-temperature; 20 µl/cm²; Gibco, Cat# 25300054), make sure that all cells have been in contact with this solution and incubate the culture flask at 37°C for approximately 4 - 5 min. Observe cell detachment under an inverted microscope. Make sure that all cells are detached (do not agitate the cells by hitting the flask, cells detach in a viscous layer) and add Trypsin-Inhibitor (20 µl/cm²; Gibco, Cat# R007100). Thereafter, resuspend the cells in growth medium (about 160 µl/cm²) and 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 having reached about 80 - 90 % confluence). Never allow the culture to become completely confluent!
Preservation:	Freezing medium: CryoStor® cell cryopreservation medium CS10 (Sigma Aldrich, Cat# C2874) Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach cells from culture vessel by using Trypsin-EDTA solution and Trypsin-Inhibitor as described above, resuspend the detached cells in growth medium and centrifuge at 170 g for 5 min. Then, 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 $0.8 – 1.4 \times 10^6$ 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: 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 its normal pH. Take a vial of frozen cells, rinse 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 - 90 % confluent at this point, they should be passaged (see subculturing).
Doubling Time:	About 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 the directive 2000/54/EG of the European Parliament and of the Council on the protection of workers from risks related to exposure to biological agents at work. 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.

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