Product-Data-Sheet for CP-MSC/TERT308

Version: September 2021

Evercyte Ord. No.:	CHT-064-0308
Designation:	CP-MSC/TERT308
Biosafety Level:	1
Shipped:	Frozen on dry ice
Growth medium:	MSC NutriStem® XF Medium (Biological Industries/SATORIUS) supplemented with G418 Final components: MSC NutriStem® XF Basal Medium (Biological Industries/SATORIUS, Cat# 05-200-1) MSC NutriStem® XF Supplement Mix (Biological Industries/SATORIUS, Cat# 05-201-1) 200 µg/ml G418 (InvivoGen, Cat# ant-gn5, ready-to-use)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Mesenchymal, spindle-shaped morphology
Source:	Placental tissue, chorionic plate
Cell Type:	Mesenchymal stem cells
Antigen Expression:	Positive for CD73, CD90, CD105, negative for CD34
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki
Comments:	CP-MSC/TERT308 was developed from human placental-derived mesenchymal stem cells (chorionic plate) by non-viral gene transfer of a plasmid carrying the hTERT gene. Positively transfected cells were selected by using neomycin phosphotransferase as selectable marker and Geneticin sulfate addition. The cells have been verified to differentiate towards mesodermal lineages. The cell line was continuously cultured for more than 50 population doublings without showing signs of growth retardation or replicative senescence. Cells readily recover from cryopreservation and no changes in growth characteristics have been observed after thawing.
Propagation:	Cells are grown on NutriCoat™ Attachment Solution in MSC NutriStem® XF Medium supplemented with G418 (see above) at 37°C in a humidified atmosphere with 5 % CO_2.

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Subculturing:	The new culture flasks have to be pre-coated with NutriCoat™ Attachment Solution (Biological Industries/SATORIUS, Cat# 05-760-1-15) following the instructions of the manufacturer. Briefly, dilute the substrate 1:500 in Ringer Solution (B. Braun Ecoflac® Plus, Cat# 33109) and transfer the diluted substrate to the culture flasks (72 µl/cm²), incubate at least 1 hours at 37°C. Before introducing cells, remove excess of coating solution. For detachment of the cells remove and discard the culture medium and wash the cells twice with PBS (each 160 µl/cm²). Remove PBS completely. Then, add CTS™ TrypLE™ Select Enzyme solution (20 µl/cm²; Gibco, Cat# A1285901), make sure that all cells have been in contact with this solution and incubate the culture flask at 37°C for approximately 2-3 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) and centrifuge at 180 g for 5 min. Discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium (about 160 µl/cm²). Then, transfer appropriate aliquots of the cell suspension to new culture vessels supplemented with growth medium (final volume of 240 µl/cm²). A split ratio of 1:6 to 1:8 twice a week is recommended (after cells have reached about 70-80 % confluence).
Preservation:	Freezing medium: CryoStor® cell cryopreservation medium CS10 (Sigma Aldrich, Cat# C2874, ready-to-use, stored at 4°C) Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach the cells (70-80 % confluence) from the culture vessel by using CTS™ TrypLE™ Select Enzyme solution as described above, resuspend the detached cells in growth medium and centrifuge at 180 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 5 x 10^5 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 CP-MSC/TERT308 cells into a T25 roux flask as described in the following:** Pre-coat a 25 cm² culture flask with NutriCoat™ Attachment Solution (see above or protocol *Passaging of CP-MSC/TERT308 cells*). Add 6 ml of growth medium to pre-coated 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. Cells should be split the day after thawing as described above or in the protocol *Passaging of CP-MSC/TERT308 cells*).
Doubling Time:	33-38 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.
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