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Product-Data-Sheet for ASC/TERT1

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Evercyte Ord. No.:	CHS-001-0005
Designation:	ASC/TERT1 (ASC52telo)
Biosafety Level:	1
Shipped:	Frozen on dry ice
Medium:	AdipoUp (Evercyte, Cat# MHS-001) EBM-2 Basal Medium 500 mL (Lonza, Cat# CC-3156) EGM-2 SingleQuot Kit Suppl. & Growth Factors (Lonza, Cat# CC-4176) but without GA-1000 and Fetal Bovine Serum 2 % Fetal Bovine Serum (Sigma Aldrich, Cat# F7524) 200 µg/mL G418 (InvivoGen, Cat# ant-gn5)
Growth:	Adherent
Organism:	Homo sapiens (human)
Morphology:	Fibroblastoid
Source:	Human adipose tissue (liposuction)
Cell Type:	Adipose tissue-derived mesenchymal stem cells
Antigen Expression:	CD73, CD90, CD105; negative for CD34
Ethical statement:	Approved by Institutional Review Board (IRB) in accordance with the Declaration of Helsinki
Comments:	ASC/TERT1 was developed from human adipose tissue-derived mesenchymal stem cells by transduction with a retroviral expression vector (pLXSN) containing the hTERT gene. The cell line was continuously cultured for more than 70 population doublings without showing signs of growth

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	retardation or replicative senescence whereas the parental cells entered senescence after having reached around 10 population doublings. Cells show expression of CD73, CD90 and CD105 and are negative for CD34. Cells can be differentiated towards adipogenic, osteogenic and chondrogenic lineages and are characterized by immunomodulatory properties.
Propagation:	Cells are grown in AdipoUp (Evercyte, Cat# MHS-001, see above) at 37°C in a humidified atmosphere with 5 % CO_2.
Subculturing:	For detachment of cells remove and discard culture medium and wash cells twice with PBS. Remove PBS completely. Then, add 0.05 % Trypsin-EDTA (1x) solution (room-temperature; 20µl/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 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 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 to 1:4 twice a week is recommended (after having reached about 80 % confluence).
Preservation:	Freezing medium: AdipoUp + 10% DMSO + 10 % FBS (Sigma Aldrich, Cat# F7524) Storage temperature: liquid nitrogen
Freezing and thawing procedure:	Freezing of cells: Detach cells from culture vessel by using Trypsin 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 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: 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 confluent at this point, they should be passaged (see subculturing).
Doubling Time:	About 36-48 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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