Designation: **hDFSC**

CLS order number: Cryovial: 300701
Vital: 330701

Origin and General Characteristics	
Organism:	Homo sapiens (human)
Ethnicity:	Caucasian
Age:	Adolescent, 16-20 years of age
Tissue:	Third molar
Morphology:	Fibroblast-like
Cell type:	Primary cells
Growth Properties:	Adherent
Description:	The DFSCs have been isolated from human Follicular tissue according to the procedure described in Ref. 1 and 2 for dental pulp cells. The adherent cellular fraction of the enzymatically digested tissue was cultured until about 70-80% confluence. After subculturing, the cells were cryopreserved in Passage 1. Consent was obtained from the donor for using this cell material for research purposes. All data were anonymised ahead of the delivery to CLS GmbH.
References:	1. Perry BC et al. Collection, Cryopreservation, and Characterization of Human Dental Pulp-Derived Mesenchymal Stem Cells for Banking and Clinical Use. Tissue Engineering: Part C, 14 (2): 149-156, 2008. 2. Woods EJ et al. Optimized Cryopreservation Method for Human Dental Pulp-Derived Stem Cells and Their Tissues of Origin for Banking and Clinical Use. Cryobiology 59 (2): 150-157, 2009.
Culture Conditions and Handling	
Culture Medium:	DMEM:Ham's F12, supplemented with L-glutamine, 15mM Hepes, and 5% fetal bovine serum. (MG-40, CLS order number 820400).
Subculturing:	Remove spent medium and rinse with PBS without calcium and magnedium. Add Accutase solution and let sit for 10 minutes at ambient temperature. Add fresh medium, dissociate the cells, and dispend into fresh culture flasks.
Seeding density:	We recommend a seeding density of about 5,000 to 10,000 cells/cm^2, once the cells have adjusted to culturing.
Fluid Renewal:	Every 2 to 3 days
Doubling time:	About 24 h
Freeze Medium:	CM-1 (CLS order no. 800150, contains serum) or CM-ACF (CLS order no. 800650, serum free)
Freezing recovery:	Fast
Sterility:	Negative for Mycoplasma contamination, as confirmed by PCR and cell-based Plasmotest.
Biosafety Level:	2 (although the current stock of hDFSC was controlled for the absence of HIV, HBV and HCV, and therefore are regarded as Biosafety level 1 material, we recommend to treat the cells according to Biosafety level 2 conditions).
Safety precautions:	If the cryovial is planned to be stored in liquid nitrogen and to be thawed in the future, special safety precautions should be followed: Protective gloves and clothing should be used and a facemask or safety goggles must be worn when transferring frozen samples into or removing from the liquid nitrogen tank. The removal of a cryovial from liquid nitrogen may result in the explosion of the frozen vial creating flying fragments.

	Caputo, J.L. Biosafety procedures in cell culture. J. Tissue Cult. Methods 11:223-227, 1988. ATCC Quality Control Methods for Cell Lines, 2nd edition, 1992.
Special Features of the Cell Line	
Applications:	Studies in Regenerative Medicine; Detection of cell surface markers; etc.

Certificate of Analysis:	The Certificate of Analysis for each batch can be requested by e-mail at service@clsgmbh.de.

Recommendations for handling of adherent cell cultures following delivery	
Cryopreserved cells	The cells come deep-frozen shipped on dry ice. Please make sure that the vial is still frozen. If immediate culturing is not intended, the cryovial(s) must be stored below -150°C after arrival. If immediate culturing is intended, please follow these instructions: Quickly thaw by rapid agitation in a 37°C water bath within 40-60 seconds. The water bath should have clean water containing an antimicrobial agent. As soon as the sample has thawed, remove the cryovial from the water bath. Note: A small ice clump should still remain and the vial should still be cold. From now on, all operations should be carried out under aseptic conditions. Transfer the cryovial to a sterile flow cabinet and wipe with 70% alcohol. Carefully open the vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of culture medium (room temperature). Resuspend the cells carefully. Centrifuge at 300xg for 3 min and discard the supernatant. The centrifugation step may be omitted, but in this case the remains of the freeze medium have to be removed 24 hours later. Resuspend the cells carefully in 10ml fresh cell culture medium and transfer them into two T25 cell culture flasks. All further steps are described in the Subculture section.
Proliferating Cultures	The cell culture flasks, 2xT25, come filled with cell culture medium. Collect the entire medium in 2x 50 ml centrifuge tubes. Carefully add 5 ml of cell culture medium to each of the two T25 cell culture flasks. Control the cell morphology and confluency under the microscope. Incubate at 37°C for a minimum of 24 hrs. Spin down the collected medium at 300x g for 3 minutes to collect the cells which may have detached during transit. If a cell pellet is visible, resuspend the cells in 5 ml of cell culture medium and transfer to 1xT25 cell culture. Incubate at 37°C for a minimum of 24 hrs.

Warranty:	CLS warrants for a high cell viability and culture performance only if the product(s) is (are) stored and cultured according to the information described above. Using cell culture media and supplements other than the ones recommended in this product information may result in satisfactory proliferation and viabilities. CLS, however, does not warrant for cell recovery, proliferation and function if differing formulations are employed.
Disclaimer:	The customer shall not be entitled to employ this product for purposes other than research. Commercial utilization shall not be permitted; in particular, the cell line, its components or materials made therefrom shall not be sold or transferred to any third party. In addition, the term ‘Commercial use’ shall mean any activity by a party for consideration and may include, but is not limited to, use of the product or its components in manufacturing, for providing services, e.g. fee for service testing, in quality control or assurance processes within the manufacturing of products for sale, for therapeutic, diagnostic or prophylactic purposes, or for resale.
Manufactured by:	CLS Cell Lines Service GmbH
This product is for research use only. Not intended for any therapeutic or diagnostic use.	