Designation: **DPSC** (Dental Pulp Stem Cells)

CLS order number: Cryovial: 300702
Vital: 330702

Origin and General Characteristics	
Organism:	Homo sapiens (human)
Age:	Adult, 18-25 years of age
Tissue:	Dental Pulp
Morphology:	Heterogenic population
Growth Properties:	Adherent
Description:	The DPSCs have been isolated from Human Pulp Tissue acc. to the procedure described in Ref. 1 and 2. The adherent cellular fraction of the enzymatically digested tissue was cultivated until about 70-80% confluence. After subculturing two times, the cells were cryopreserved in Passage 3.

Culture Conditions and Handling	
Culture Medium:	DMEM:Ham's F12, supplemented with L-glutamine, 15mM Hepes, and 5% fetal bovine serum.
Subculturing:	Remove spent medium and rinse with PBS without calcium and magnedium. Add Accutase solution and let sit for 10 minutes at 37°C. Add fresh medium, dissociate the cells, and dispend into fresh culture flasks. We recommend a seading 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
Freeze Medium:	CM-1 (CLS order no. 800150, contains serum) or CM-2 (CLS order no. 800250, serum free)

Thawing and Plating Protocol

Remove the vial from Liquid Nitrogen. Short term storage on dry ice ahead of thawing is adviced.

Quickly thaw in a 37°C water bath with constant, rapid agitation within 40-60 seconds. The water bath should have clean water containing an antimicrobial agent. As soon as only a small piece of ice is visible within the vial, remove it from the water bath.

From now on, all operations should be carried out under aseptic conditions:

Wipe the vial with 70% alcohol and transfer it into a sterile flow cabinet.

Carefully open the vial and transfer the cell suspension into a sterile 15 ml centrifuge tube containing 8 ml of prewarmed cell culture medium.

Resuspend the cells carefully.

Centrifuge at 200xg for 3 min and remove the supernatant. Resuspend the cells carefully in 5 ml of prewarmed cell culture medium and transfer them into a T-25 (25 cm^2) cell culture flask. (The centrifugation step may be omitted, but in this case the remains of the freeze medium have to be removed 24 hours later).

Observe the cells about 24 hrs later; the cells should have adhered to the bottom of the T-25.

At about 70% confluence the cells are ready to be subcultured at a density of 5x10^3 to 1x10^4 cells/cm^2.

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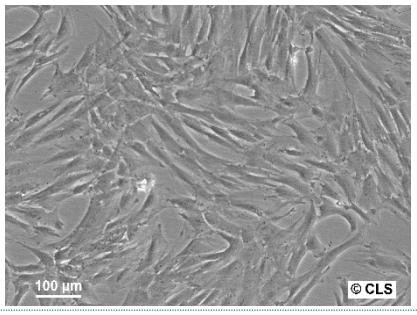
Special Features of DPSCs and Recommended Use	
Photo copyright by CLS GmbH.	
Applications:	Studies in Regenerative Medicine; Detection of cell surface markers; etc.

Notes:

Although this material has been tested thoroughly, the user should treat it as potential biohazardous. Protective clothing and eyewear are strongly recommended.

Consent was obtained by 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.

This product is for research use only. Not intended for any therapeutic or diagnostic use.

General recommendations for handling of adherent cell cultures following delivery

Cryopreserved cells

If immediate culturing is not intended, the cryovial(s) should be stored in liquid nitrogen after arrival.

Proliferating Cultures

The cell culture flasks are completely filled with cell culture medium to prevent loss of cells during transit.
Remove the entire medium except for a sufficient volume to cover the floor of the flask.
Incubate at 37°C for 24 hrs.
Sometimes the cultures are handled roughly during transit, and most of the cells detach and float in the culture medium. If this has occurred, remove the entire content of the flask and centrifuge at 200xg for 3 minutes. Discard the supernatant, resuspend the cells in 5 ml of culture medium and transfer the entire cell suspension into cell culture flasks of suitable size.

Safety precautions for frozen cell lines

If the cryovial is planned to be stored in liquid nitrogen and to be thawed in the future, special safety precautions should be followed:

- Wearing protective gloves and clothing as well as safety goggles is recommended when storing and/or thawing the cryovial.
- The removal of a cryovial from liquid nitrogen can result in the explosion of the cryovial creating flying fragments.

References: 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.

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