**Designation:** **HMSC.WJ-500**

**Human Mesenchymal Stem Cells (Wharton's Jelly-derived)**

CLS order number: Cryovial: 300685

| Origin and General Characteristics | |
|---|---|
| Organism: | Human (Homo sapiens) |
| Tissue: | Wharton's Jelly |
| Cell type: | Mesenchymal Stem Cells |
| Growth Properties: | Adherent |
| Description: | Human Mesenchymal Stem Cells (hMSC) are primary cells which can be successfully cultured for approximately five to six passages. |
| **Culture Conditions and Handling** | |
| Culture Medium: | MSC expansion media. Antibiotics or antimycotics should not be used as an alternative to proper aseptic technique. |
| Thawing and passing cells: | Remove the vial of cells from dry ice. Defrost the vial in a 37°C water bath with constant, moderate agitation, until ice in the ampoule is no longer visible.<br>Continue to warm the ampoule in the water bath for 30 seconds with gentle agitation.<br>Immediately disinfect the vial with 70% isopropanol.<br>Working in a laminar flow hood, open the vial and transfer the contents to a sterile 15ml tube.<br>Add approx. 10 ml of complete MSC Expansion media which has been prewarmed to 37°C.<br>Centrifuge the suspended cells at 200xg for 10 minutes.<br>Decant the medium and re-suspend the pellet gently in 10 ml of complete MSC Expansion media, then transfer into a T25 (25 $cm^2$) cell culture flask.<br>Count the cells using a hematocytometer of cell counter, then place the flask in an incubator at 37°C / 5% $CO_2$ / 90% humudity.<br>Cells will be ready to pass between 3-7 days. Cells should be subcultured at a density of 5,000 to 10,000 cells/$cm^2$ or desired plating density. |
| Fluid Renewal: | 2-3 times weekly |
| Sterility: | Fluorescence (DAPI) test: negative; Mycoplasma specific PCR: negative; Bacteria specific PCR: negative |
| **Certificate of Analysis** | |
| The cells are tested for HIV-1, HIV-2, Hepatitis B and Hepatitis C using sensitive PCR based assays and are negative for these viruses. Nevertheless, all human cells must be used in accordance with established laboratory safety procedures and only under supervision of trained personnel. | |
| HMSC.WJ-cells are evaluated by flow cytometry for specific stem cell markers, such as CD29(+), CD44(+), CD90 (+), CD105 (+), CD14(-) and CD45(-). | |
| | |
| Key References: | |
| Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ, Fu YS, Lai MC, Chen CC. Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. Stem Cells 22(7): 1330-7, 2004.<br>Mitchell KE, Weiss ML, Mitchell BM, Martin P, Davis D, Morales L, Helwig B, Beerenstrauch M, Abou-Easa K, Hildreth T, Troyer D, Medicetty S. Stem Cells 21(1): 50-60, 2003. | |

Manufactured by: Cellular Engineering Technologies (CET) Inc., USA

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

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## Recommendations for handling of cryopreserved cells following delivery

If immediate culturing is not intended, the cryovial(s) must be stored in liquid nitrogen (-196°C) or at least at -80°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.

**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:

- Protective gloves and clothing should be used and a facemask or safety goggles must be worn 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.