Designation: **MOLT-3**

CLS order number: Cryovial: 300116
Vital: 330116

Origin and General Characteristics		
Depositor:	Homo sapiens (human)	
Tissue:	Blood	
Age:	19 years old	
Gender:	Male	
Morphology:	Lymphoblast	
Cell type:	T lymphoblast (ALL, acute lymphoblastic leukaemia)	
Growth Properties:	Suspension	
Description:	The T-cell lines MOLT-3 and MOLT-4 are derived from the leukemic cells of a patient with acute lymphoblastic leukaemia whilst in relapse. The cells should be handled under laboratory containment level 2 conditions.	
References:	Minowada J et al. Rosette-forming human lymphoid cell lines. I. Establishment and evidence for origin of thymus-derived lymphocytes. J Natl Cancer Inst 49: 891-5, 1972. Huang CC, Woods LK, Moore GE, Minowada J. Cytogenetic study of human lympoid T-cell lines derived from lymphocytic leukemia. J Natl. Cancer Inst 53: 655, 1974. Greenberg JM, Gonzalez-Sarmiento R, Arthur D, Wilkowski CW, Streifel BJ and Kersey JH. Immunophenotypic and Cytogenetic Analysis of Molt-3 and Molt-4: Human T-Lymphoid Cell Lines with Rearrangement of Chromosome 7. Blood 72: 1755-1760, 1988.	
Culture Conditions and Handling		
Culture Medium:	RPMI 1640 with sodium bicarbonate, L-glutamine, 1mM sodium pyruvate and 10% FBS	
Subculturing:	Maintain cultures between 5 x 10^5 to 6 x 10^5 cells/ml. Incubate at 5% CO_2, 37°C until a maximum cell density of 1-2 x 10^6 cells/ml is achieved.	
Seeding density:	0,5 – 1 x 10^5/ml	
Freeze Medium:	CM-1 (CLS order number: 800125, 25ml, 800150, 50ml)	
Freezing recovery:	24-48 hrs	
Sterility:	Fluorescence (DAPI) test: negative; Mycoplasma specific PCR: negative; Bacteria specific PCR: negative	
Biosafety Level:	2	
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		
Viruses:	SMRV: Negative, as confirmed by Real-Time PCR	
Karyotype:	Hypertetraploid	
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12,13 D13S317: 12,13	D18S51: 12,13,16,17 Penta E: 14,16 Penta D: 8,13

	D16S539: 11,14,15 D5S818: 12,13 D7S820: 7,8,9,10 THO1: 6,8 TPOX: 8 vWA: 17,18 D3S1358: 15,16,17 D21S11: 29,30,31,32	D8S1179: 9,13,14,15 FGA: 19,21,25 D1S1656: 15.3,16,16.3 D6S1043: 14,15,16 D2S1338: 23,24 D12S391: 17,19,20 D19S443: 14,15,16
Antigen Expression:	CD1(+); CD5(+); CD7(+); CD11a(+) (Greenberg et al. 1988).	

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

Recommendations for handling of cells growing in suspension 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 one T25 cell culture flask. All further steps are described in the Subculture section.
Proliferating Cultures	The cell culture flask, 1xT25, comes filled with cell culture medium. Incubate at 37°C for a minimum of 24 hrs. Count the cells, spin down the cell suspension at 300x g for 3 minutes to collect the cells. Resuspend the cells in an appropriate amount of fresh cell culture medium and transfer to new cell culture flasks. Incubate at 37°C for a minimum of 24 hrs.
Recommendations for handling of suspension 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.