**Designation:** MOLT-4  
**CLS order number:** Cryovial: 300115  
Vital: 330115

### Origin and General Characteristics
- **Organism:** Homo sapiens (human)
- **Age:** 19 years
- **Gender:** Male
- **Tissue:** Blood
- **Morphology:** Lymphoblast
- **Cell type:** T lymphoblast (ALL, acute lymphoblastic leukemia)

### Growth Properties:
- **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 do not produce immunoglobulin or Epstein-Barr virus (Minowada, 1972). There is a G -> A mutation at codon 248 of the p53 gene; P53 is not expressed (Rodrigues, 1990).

### Culture Conditions and Handling
- **Culture Medium:** RPMI 1640 medium supplemented with 4.5g/L glucose, 2 mM L-glutamine and 10% fetal bovine serum (MG-72, CLS order number 820702).
- **Subculturing:** Subculture by diluting an aliquot of the cell suspension in fresh medium inside a new cell culture flask. Start new cultures at 4 x 10^5 cells/ml. The cell density should not exceed 2 x 10^6 cells/ml.
- **Fluid Renewal:** Add fresh medium every 2 to 4 days (depending on cell density)
- **Freeze Medium:** CM-5 (CLS order number: 800525, 25ml, 800550, 50ml)
- **Sterility:** Fluorescence (DAPI) test: negative; Mycoplasma specific PCR: negative; Bacteria specific PCR: negative
- **Biosafety Level:** 1

### Special Features of the Cell Line
- **Viruses:** SMRV: Negative, as confirmed by Real-Time PCR
- **Karyotype:** Hypertetraploid; modal number: 96; two X and two Y chromosomes.
- **DNA Profile (STR):**
  - Amelogenin: X,Y
  - CSF1PO: 11,12
  - D1S317: 12,13
  - D16S539: 11,14
  - D5S818: 12
  - D7S820: 8,10,11
  - THO1: 6,8
  - TPOX: 8
  - vWA: 17,18
  - D3S1358: 15,16
  - D21S11: 28,29,30
  - D18S51: 12,13,17
  - Penta E: 14,15,16
  - Penta D: 8,12,13
  - D8S1179: 9,13,14
  - FGA: 22,24
- **Antigen Expression:** CD1 (49%), CD2 (35%), CD3 A (26%) B (33%) C (34%), CD4 (55%), CD5 (72%), CD6 (22%), CD7 (77%)
- **Protein Expression:** p53 positive
- **Products:** High levels of terminal deoxynucleotidyl transferase (TdT) are produced

### References:
Recommendations for handling of suspension cells following delivery

Cryopreserved cells
If immediate culturing is not intended, the cryovial(s) may be stored in liquid nitrogen 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 (a small ice clump should remain and the cryovial should still be cold).
From now on, all operations should be carried out under aseptic conditions.
Immediately 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. Resuspend the cells carefully. The cells may be spun down at 250xg for 3 minutes (this depends on the cell line used). After centrifuging, aseptically remove the supernatant and add 10 ml of fresh cell culture media. Carefully resuspend the cells and distribute into one 25cm² cell culture flask. Incubate at 37°C/5% CO₂.
Subculture as soon as the cell concentration has reached 1 x 10⁶ cells/ml. It is recommended to distribute the cells into new flasks containing fresh medium thus diminishing the amount of dead cells and cell debris. Adjust to a cell concentration of 1-2 x 10⁵ cells/ml depending on the specification given for the cell line. After about 1-2 times of sub-culturing as recommended the percentage of viable cells should be > 90%.

Proliferating Cultures
Immediately after receipt the cell concentration should be determined. If the cell concentration already has reached a value of 1 x 10⁶ cells/ml or even more, subculture the cells as described above. Remove the entire content of the flask and centrifuge at 300xg for 10 minutes.
Resuspend the cell pellets as suggested under subculture procedures described on the appropriate datasheet.

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.