Designation: **HuT-78**

CLS order number: Cryovial: 300338
Vital: 330338

### Origin and General Characteristics

**Depositor:** T. Lindl  
**Organism:** Homo sapiens (human)  
**Ethnicity:** Caucasian  
**Age:** 53 years  
**Gender:** Male  
**Tissue:** Blood (cutaneous lymphoma)  
**Morphology:** Lymphoblast  
**Cell type:** T lymphocyte  
**Growth Properties:** Suspension  
**Description:** Derived from the peripheral blood of a patient with Sézary syndrome. The line has the properties of a mature human T cell with helper/inducer activity. The growth rate is stimulated by IL-2. TNF alpha is an autocrine growth factor for Hut-78.

### Culture Conditions and Handling

**Culture Medium:** RPMI 1640 medium supplemented with 4.5g/L glucose, L-glutamine, and 10% fetal bovine serum (MG-72, CLS order number 820702).  
**Subculturing:** Start cultures at 1 x 10^5 cells/ml and maintain between 1 x 10^5 and 1 x 10^6 cells/ml. Subculture by pipetting aliquots into new cell culture flasks containing the appropriate amount of cell culture media.  
**Freeze Medium:** CM-1 (CLS order number 800125, 25ml; 800150, 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  
**DNA Profile (STR):**  
<table>
<thead>
<tr>
<th>Marker</th>
<th>Alleles</th>
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<tbody>
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<td>D7S820: 8,11</td>
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<tr>
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<td>vWA: 14,15</td>
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<td>D21S11: 30</td>
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<td>FGA: 21,25</td>
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</table>

**Antigen Expression:** CD4  
**Receptors Expressed:** interleukin-2 (interleukin 2, IL-2)  
**Products:** interleukin-2 (interleukin 2, IL-2); tumor necrosis factor alpha (TNF alpha)  
**Protein Expression:** p53 negative

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.