Human T cell leukemia LOUCY

A Cells, Media and Reagents Information

Company Name	Catalog No	Name	Contain
DSMZ	ACC 394	LOUCY	5 E+06 cells in 40% medium, 50% FBS, 10% DMSO
Invitrogen	21870-076	RPMI 1640 Base medium	500 ml
Invitrogen	15140-122	liquid penicillin-streptomycin	10 units/ul of penicillin and 10 µg/ul streptomycin
Invitrogen	35050-079	Glutamax	200 mM
Atlas Biologicals	F-0500-A	FCS	500 ml

B Preparation of Media

1 Decontaminate the external sufraces of all supplement vials and the medium bottle with 70% ethanol .

2 Aseptically open each supplement vial and add the following reagents to the base medium with a pipette:
- Penicillin, final concentration 100 Unit/mL
- Streptomycin, final concentration 100 µg/mL
- Glutamax, final concentration 20 mM
- FCS to 10%

3 Rinse each cryovial with the medium.

4 Record the expiration date (one month from the preparation date) on the medium bottle.

C Thawing of Cells / Initiation of Culture Process

1 Recommended seeding density for Loucy celsl are 5 E+05 cells / ml .

2 Determine the total number of flasks by following equation.

Total # of flasks = Total Cell Count x Percent Viability x Seeding Efficiency / (Growth Area x Rec. Seeding Density)

3 Add 15 ml medium to T75 (1 ml / 5 cm2) to equilibrate at 37C, CO2, 5% for 30 min.

4 Quickly thaw the cryovial in a 37C water bath. (Do not submerge it and remove it as soon as the ice melts)

5 Resuspend cells in cryovial using a micropipette and transfer to the T75 set up earlier,.

6 Gently rock T75 then place it back into incubator.

Note Centrifugation should not be performed because centrifugation is more damaging than residual DMSO in the culture.

D Subculturing and Maintenance

1 Subculture when cells are at 1.5 E+06 cells/mL after 2 to 3 days growth.

2 Aliquot stated volume medium and reagents as listed below and warm to room temperature.

Cell Growth Vessels	T75 Flask	T175 Flask	150 mm Dish
TNS	5 ml	10 ml	8 ml
Medium	35 ml	70 ml	52 ml

The following instructions are for a T75 falsk. Adjust all volumes accordingly for other size culture vessels.

3 Mix cell suspension with a 10 mL pipet 3 times, then aspirate medium from the culture vessel and transfer into a 50 mL conical

4 Centrifuge at 220 xg for 5 min at RT to pellet the cells.

5 Aspirate most supernatant, except for 100 - 200 ul, and flick the tube with finger to loosen pellet.

6 Resuspend cells with 5 ml to 10 ml medium and mix with 5 ml or 10 ml pipet to ensure a uniform suspension.

7 Determine cell number and viability (if necessary more dilute cells with HEPES-BSS to count)

8 Determine the total number of flasks to inoculate by using the following equation.

9 Total # of flasks to innoculate = Total # of viable cells / (Growth area x Rec. seeding Density)

10 Transfer the appropriate amount of growth medium (1 ml / 5 cm2) to the new vessels and warm in incubator for 30 min.

11 Resupend cells with 5 ml or 10 ml pipet about 10 time to make sure cells seperated each other very will.

12 Dispense the calculated volume into the prepared subculture falsks.

13 Place the new culture vessels back into a 37C humidified incubator with 5% CO2 .

14 Change medium every other day.

E Large Scale Harvest (> 2E+08 cells)

1 Thaw 1 Cryovial of cells [> 5 E+05 cells / Amp] and plate into two T75 flasks .

2 Change fresh medium next day.

3 Check cell confluence every day. When cells are 60% ~ 80% confluent (need 8 to 10 days growth),
subculture cells (as described above under subculturing) into new vessels.
Each T75 flask can yield ~ 6 E+07 cells.

4 Count cells with hemocytometer and seed as recommended seeding density (5 E+05 cells/mL) into needed number T175 flasks
Total number of flasks depends upon cell yield and seeding density.

5 Subculture cells 3 or 4 more times until the desired cell number (> 5 E+07 cells) is achieved for final harvesting (> 2 E+08 cells).

6 Subculture when these flasks have reached 80% confluence. Each T175 flask can yield ~ 1.2 E+08 cells.
Seed cells as recommended seeding density (5 E + 05 cells/mL) into needed # of 150 mm dishes.
(can be up to 60 x 150 mm dishes)

7 When 60% ~ 80% confluent (need ~ 7 days) harvest all cells (> 2 E+08 cells).

Each 150 mm dish can yeild ~ 9 E+07cells.

F Morphology

round, rather small cells growing singly in suspension