

Human T cell leukemia DND41

A Cells, Media and Reagents Information

Company Name	Catalog No	Name	Contain
DSMZ	ACC 525	DND41	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 surfaces 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 DND41 cells are 5 E+05 cells / ml .
 - 2 Determine the total number of flasks by following equation.
$$\text{Total \# of flasks} = \text{Total Cell Count} \times \text{Percent Viability} \times \text{Seeding Efficiency} / (\text{Growth Area} \times \text{Rec. Seeding Density})$$
 - 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 flask. 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.
$$\text{Total \# of flasks to inoculate} = \text{Total \# of viable cells} / (\text{Growth area} \times \text{Rec. seeding Density})$$
- 9 Transfer the appropriate amount of growth medium (1 ml / 5 cm²) to the new vessels and warm in incubator for 30 min.
- 10 Resuspend cells with 5 ml or 10 ml pipet about 10 times to make sure cells separated each other very well.
- 11 Dispense the calculated volume into the prepared subculture flasks.
- 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 4 to 6 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 1 or 2 more times until the desired cell number (> 5 E+ 07 cells/mL 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 ~ 4 days) harvest all cells (> 2 E+08 cells).
Each 150 mm dish can yeild ~ 9 E+07 cells.

F Morphology

Morphology: round cells growing singly or, occasionally, in clumps