Human T cell leukemia DND41

A Cells, Media and Reagents Information

<table>
<thead>
<tr>
<th>Company Name</th>
<th>Catalog No</th>
<th>Name</th>
<th>Contain</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSMZ</td>
<td>ACC 125</td>
<td>DND41</td>
<td>5 E+06 cells in 40% medium, 50% FBS, 10% DMSO</td>
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<tr>
<td>Invitrogen</td>
<td>21870-076</td>
<td>RPMI 1640 Base medium</td>
<td>500 ml</td>
</tr>
<tr>
<td>Invitrogen</td>
<td>15140-122</td>
<td>liquid penicillin-streptomycin</td>
<td>10 units/ul penicillin and 10 µg/ul streptomycin</td>
</tr>
<tr>
<td>Invitrogen</td>
<td>39250-079</td>
<td>Glutamax</td>
<td>200 mM</td>
</tr>
<tr>
<td>Atlas Biologicals</td>
<td>F-0500-A</td>
<td>FCS</td>
<td>500 ml</td>
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B Preparation of Media

1. Decontaminate the external surfares 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.
   \[
   \text{Total # of flasks} = \frac{\text{Total Cell Count} \times \text{Percent Viability} \times \text{Seeding Efficiency}}{(\text{Growth Area} \times \text{Rec. Seeding Density})}
   \]
3. Add 15 ml medium to T75 (1 ml / 5 cm²) to equilibrate at 37°C, CO₂, 5% for 30 min.
4. Quickly thaw the cryovial in a 37°C 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.

<table>
<thead>
<tr>
<th>Cell Growth Vessels</th>
<th>T75 Flask</th>
<th>T175 Flask</th>
<th>150 mm Dish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>35 ml</td>
<td>70 ml</td>
<td>8 ml</td>
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</table>

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.
9. Total # of flasks to inoculate = \( \frac{\text{Total # of viable cells}}{(\text{Growth area} \times \text{Rec. Seeding Density})} \)
10. Transfer the appropriate amount of growth medium (1 ml / 5 cm²) to the new vessels and warm in incubator for 30 min.
11. Resuspend cells with 5 ml or 10 ml pipet about 10 times to make sure cells separated each other very well.
12. Dispense the calculated volume into the prepared subculture flasks.
13. Place the new culture vessels back into a 37°C 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
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)
When 60% - 80% confluent (need ~ 4 days) harvest all cells (> 2 E+08 cells).
Each 100 mm dish can yield ~ 9 E+07 cells.

**Morphology**

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