Adherent

Incubate 37°C in a 5% CO2 atmosphere. (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO2 in air atmosphere. (Table 1) and incubate for 2 minutes. Gently tapping the flask, observe cells under an inverted microscope. Cells usually detach in 2 to 3 minutes. (Table 1) and incubate for 2 minutes. Gently tapping the flask, observe cells under an inverted microscope. Cells usually detach in 2 to 3 minutes.

Briefly rinse the cell layer with 1XPBS (SCRR-2201) solution to remove all traces of serum, which may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

Remove and discard culture medium.

To insure the highest level of viability, be sure to warm media to 37°C before using it on the cells.

To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 15%
- 800.638.6597 or 703.365.2700
- Email: Tech@atcc.org

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5. Transfer all cells into a centrifuge bottle or tube and centrifuge at 270 x g for 5 minutes.
6. Remove and discard the supernatant.
7. Add 10 mL complete growth medium to the cell pellet and with 10 mL pipette resuspend the cells gently (create a single-cell suspension).
8. Add more complete growth medium (Table 1) to the cell suspension as needed to plate cells at approximately $0.8 \times 10^4$ cells/cm$^2$.
9. Place flasks in the incubator @ 37°C with a 5% CO$_2$ in air atmosphere.

<table>
<thead>
<tr>
<th>Flask/Plate</th>
<th>Growth Area (cm$^2$)</th>
<th>1xPBS (mL)</th>
<th>Trypsin/EDTA (mL)</th>
<th>Equal vol. Complete Growth Medium (mL)</th>
<th>Growth Medium (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T225</td>
<td>225</td>
<td>6 ± 0.2</td>
<td>6 ± 0.2</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>75</td>
<td>5 ± 0.1</td>
<td>3 ± 0.1</td>
<td>3 ± 0.1</td>
<td>12</td>
</tr>
<tr>
<td>T25</td>
<td>25</td>
<td>3 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>6</td>
</tr>
<tr>
<td>6 well</td>
<td>9.5</td>
<td>1 ± 0.1</td>
<td>1 ± 0.1</td>
<td>1 ± 0.1</td>
<td>3</td>
</tr>
</tbody>
</table>

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 5th edition, published by Alan R. Liss, N.Y., 2005.

Subcultivation Ratio: 1:5 to 1:8

Cryopreservation Medium
Complete growth medium, supplemented with an additional 40% FBS and 10% DMSO(v/v)
Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

References
References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 1
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the Biosafety in Microbiological and Biomedical Laboratories from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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