Preparing 48-Well Plate Feeders

In preparing 48-well plates, a dilution must be made from the regular density of 3.5 x 10^5 cells/ml. The required density for 48-well plates is: 8.2 x 10^4 cells/ml (650 ml/well or 31.2 mls/plate).

Example:

For a total of 5 x 48-well plates, the calculation is:

5 plates x 31.2 mls = 156 mls + 10 mls Extra = 166 mls (Total)

166 mls x 8.2 x 10^4 cells/ml = 1.36 x 10^7 cells (Total)

1.36 x 10^7 cells : 3.5 x 10^5 cells/ml = 38.86 mls

Dilution: 38.9 mls (of cell suspension @ 3.5 x 10^5 cells/ml) + 127.1 mls Media.

Take a count at this point, to verify the cell density of 8.2 x 10^4 cells/ml.
Plate-out this feeder media (dilution) into the 48-well plates.
REMEMBER TO USE PLATES THAT HAVE BEEN GELLED, 2 HOURS PRIOR THIS.
Dispense 650 ml of feeder media/well (31.2 mls/plate).
Mix plates to distribute cells uniformly and incubate.

<table>
<thead>
<tr>
<th>No. of Plates</th>
<th>Total # of Cells</th>
<th>Dilution (mls)</th>
<th>Total Vol. (mls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 plates</td>
<td>1.36 x 10^7 cells</td>
<td>38.9 + 127.1</td>
<td>166 mls</td>
</tr>
<tr>
<td>10 plates</td>
<td>2.64 x 10^7 cells</td>
<td>75.0 + 247.0</td>
<td>322</td>
</tr>
<tr>
<td>15 plates</td>
<td>3.92 x 10^7 cells</td>
<td>112.0 + 366.0</td>
<td>478</td>
</tr>
<tr>
<td>20 plates</td>
<td>5.20 x 10^7 cells</td>
<td>148.5 + 485.5</td>
<td>634</td>
</tr>
</tbody>
</table>

Feeders/STO Media: DMEM, 7% FCS, 1 X GPS.

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