DAY 0: Cell seeding
→ Seed cells in \( V \) mL of serum containing medium according to the table below

Quantities per well, dish or flask

<table>
<thead>
<tr>
<th>Culture vessel</th>
<th>Number of cells*</th>
<th>( V ) = volume of medium during transfection</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well</td>
<td>2 500 – 7 500</td>
<td>0.2 mL</td>
</tr>
<tr>
<td>24-well</td>
<td>15 000 – 35 000</td>
<td>1 mL</td>
</tr>
<tr>
<td>12-well</td>
<td>30 000 – 70 000</td>
<td>2 mL</td>
</tr>
<tr>
<td>6-well / 35 mm</td>
<td>100 000 – 200 000</td>
<td>4 mL</td>
</tr>
<tr>
<td>100 mm / flask 75 cm²</td>
<td>750 000 – 1.25 x 10⁶</td>
<td>15 mL</td>
</tr>
</tbody>
</table>

*For suspension cells, please refer to the complete protocol.

DAY 1: Transfection = 1 nM siRNA
→ Perform transfection in the presence of serum
→ Transfect cells at 30-50% confluency

Dilute \( X \) pmoles of siRNA in \( W \) \( \mu \)L of medium without serum
Vortex 10 s and spin down

Add \( Y \) \( \mu \)L of INTERFERin® reagent
Vortex 10 s, spin down and incubate 10 min at RT

Add transfection mix to the cells in serum containing medium
During the incubation time, remove \( Z \) mL of serum containing medium

Incubate 24 to 72 h

Quantities per well, dish or flask

<table>
<thead>
<tr>
<th>Culture vessel</th>
<th>( W ) = volume of medium without serum</th>
<th>( X ) = amount of siRNA added (1 nM)</th>
<th>( Y ) = volume of INTERFERin® reagent</th>
<th>( Z ) = volume of serum containing medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well</td>
<td>50 ( \mu )L</td>
<td>0.17 pmoles (2.4 ng)</td>
<td>0.75 ± 0.5 ( \mu )L</td>
<td>0.125 mL</td>
</tr>
<tr>
<td>24-well</td>
<td>100 ( \mu )L</td>
<td>0.6 pmoles (8.4 ng)</td>
<td>2 ± 1 ( \mu )L</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>12-well</td>
<td>200 ( \mu )L</td>
<td>1.2 pmoles (17 ng)</td>
<td>4 ± 2 ( \mu )L</td>
<td>1 mL</td>
</tr>
<tr>
<td>6-well / 35 mm</td>
<td>200 ( \mu )L</td>
<td>2.2 pmoles (31 ng)</td>
<td>8 ± 4 ( \mu )L</td>
<td>2 mL</td>
</tr>
<tr>
<td>100 mm / flask 75 cm²</td>
<td>500 ( \mu )L</td>
<td>10.5 pmoles (147 ng)</td>
<td>40 ± 10 ( \mu )L</td>
<td>10 mL</td>
</tr>
</tbody>
</table>

DAY 2-3: Analyze gene silencing

See back page for optimization tips
Protocol Optimization

✦ The siRNA final concentration may range from 1 to 50 nM depending on the cells and the target gene.
✦ Cell confluency: between 30 and 50% at the time of transfection.
✦ Check our online Cell Transfection Database at:
  http://www.polyplus-transfection.com/resources/cell-transfection-database/

Tips to increase cell viability of sensitive cells

✦ Replace medium 4 h after transfection.
✦ Check that silencing the target gene does not affect cell viability.

Use appropriate controls

✦ Positive control: housekeeping gene (GAPDH or HPRT).
✦ Negative control: mismatch, scramble or non-targeting sequence.
✦ Be cautious with fluorescently labeled siRNA: 20 to 30 nM are needed to detect a signal, while only 1 nM can be sufficient for efficient silencing using INTERFERin®.

Good siRNA Transfection Practices

✦ Store appropriately INTERFERin® (5 ± 3°C). Do NOT freeze INTERFERin®.
✦ Ensure that cells have been passaged more than twice and less than 20 times prior to transfection.
✦ Discard overconfluent cells.
✦ Check the half-lives of the protein and mRNA of interest and measure gene silencing accordingly 24 to 96 h after transfection.
✦ Regularly check for mycoplasma contaminations.
✦ Serum quality may drastically affect transfection efficiency. When purchasing a new batch of serum or trypsin, check cell viability as well as transfection efficiency.

Note: INTERFERin® is recommended for siRNA transfection. Please refer to the complete protocol available online at: http://www.polyplus-transfection.com/resources/product-literature/. Please use jetPRIME® for DNA/siRNA cotransfection.