**jetPRIME® transfection reagent**

**Short protocol – siRNA Transfection**

**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 growth medium for cell seeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-well</td>
<td>25 000 - 40 000</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>12-well</td>
<td>50 000 - 80 000</td>
<td>1 mL</td>
</tr>
<tr>
<td>6-well / 35 mm</td>
<td>100 000 - 150 000</td>
<td>2 mL</td>
</tr>
<tr>
<td>100 mm / flask 75 cm²</td>
<td>( 0.5 \times 10^6 ) - ( 1 \times 10^6 )</td>
<td>10 mL</td>
</tr>
</tbody>
</table>

**DAY 1: Transfection**

→ Perform transfection in the presence of serum
→ Use jetPRIME® buffer only
→ Transfect cells at 50% confluency

Dilute \( X \) pmoles of siRNA in \( W \) µL of jetPRIME® buffer
Vortex 10 s and spin down

Add \( Y \) µL of jetPRIME® reagent

Vortex 1 s, spin down and incubate 10 min at RT

Add transfection mix to the cells in serum containing medium

**Quantities per well, dish or flask**

<table>
<thead>
<tr>
<th>Culture vessel</th>
<th>( W ) = volume of jetPRIME® buffer</th>
<th>( X ) = amount of siRNA added (10nM)</th>
<th>( X ) = amount of siRNA added (50nM)</th>
<th>( Y ) = volume of jetPRIME® reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-well</td>
<td>50 µL</td>
<td>5.5 pmoles (76 ng)</td>
<td>27.5 pmoles (381 ng)</td>
<td>2 µL</td>
</tr>
<tr>
<td>12-well</td>
<td>100 µL</td>
<td>11 pmoles (152 ng)</td>
<td>55 pmoles (762 ng)</td>
<td>3 µL</td>
</tr>
<tr>
<td>6-well / 35 mm</td>
<td>200 µL</td>
<td>22 pmoles (306 ng)</td>
<td>110 pmoles (1524 ng)</td>
<td>4 µL</td>
</tr>
<tr>
<td>100 mm / flask 75 cm²</td>
<td>500 µL</td>
<td>105 pmoles (1460 ng)</td>
<td>525 pmoles (7274 ng)</td>
<td>20 µL</td>
</tr>
</tbody>
</table>

**DAY 2-3: Measure gene expression**

*See back page for optimization tips*

*Download complete protocol on [http://www.polyplus-transfection.com/resources/product-literature/*]

Contact us:
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Web site: www.polyplus-transfection.com
Protocol Optimization

- Check our online Cell Transfection Database for cell specific protocols at:
- Test different siRNA concentration ranging from 10 to 50 nM (final concentration).
- Use cells at 50% confluency at time of transfection.

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) or fluorescently labeled siRNA.
- Negative control: mismatch, scramble or non-targeting sequence.

Good siRNA Transfection Practices

- Store appropriately jetPRIME® (5 ± 3°C).
- 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: jetPRIME® is also recommended for DNA transfection, virus production and DNA/siRNA cotransfection, please refer to the complete protocol available online at: