**PepMute™ siRNA Transfection Reagent**

--- A Standard Protocol for siRNA Transfection of Mammalian Cells

**Introduction:**
PepMute™ siRNA Transfection Reagent is a novel peptide-based siRNA delivery tool that provides more than 95% silencing efficiency at 1 nM siRNA in a variety of mammalian cells. With our proprietary peptide simulation technology (PST), PepMute™ reagent was identified and validated as an exceptionally efficient vector for condensing and transfecting short (under 100 bp) single or double stranded nucleic acids such as siRNA, miRNA mimics and DNA oligoes to wide spectrum of mammalian cells.

**Important Guidelines for Transfection:**
- PepMute™ reagent was formulated as a powerful siRNA delivery tool. For most adherent cell lines and primary cells, siRNA at ~5 nM is basically sufficient to obtain up to 90% gene silencing, as observed for Hela, MCF and NIH-3T3. For hard-to-transfect cells, we recommend using a final siRNA concentration of 30 nM.
- While the standard protocols for siRNA transfection and siRNA/DNA co-transfection are being given below, optimization is often needed for maximal gene silencing.

**PART I. Standard siRNA Transfection of Adherent Cells**

**Step I. Preparation of Working Solution of PepMute™ Transfection Buffer:**
PepMute™ Transfection Buffer (5x) is provided as 5x concentrated stock solution. To make working solution, dilute one part of the stock solution with 4 parts of ddH₂O into a sterile bottle. The working solution (1x) is stable at RT for 24 months.

**Note:** Always keep PepMute™ Transfection Buffer (5x) at RT. If refrigerated, white precipitates may appear. It won’t affect the transfection efficiency. After dilution with 4 parts of ddH₂O, the working solution (1x) is stable at RT for 24 months.

**Step II. Cell Seeding:**
Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches the optimal ~50% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30–60 min before transfection.

**Note:** PepMute™ reagent is NOT interfered by serum and antibiotics, therefore serum and antibiotic containing medium can be used during the entire experiment.

**PART II. A Standard Protocol for DNA/siRNA Co-transfection**

**Step I. Preparation of Working Solution of PepMute™ Transfection Buffer:**
PepMute™ Transfection Buffer (5x) is provided as 5x concentrated stock solution. To make working solution, dilute one part of the stock solution with 4 parts of ddH₂O into a sterile bottle. The working solution (1x) is stable at RT for 24 months.

**Note:** Always keep PepMute™ Transfection Buffer (5x) at RT. If refrigerated, white precipitates may appear. It won’t affect the transfection efficiency. After dilution with 4 parts of ddH₂O, the working solution (1x) is stable at RT for 24 months.
of ddH₂O to make PepMute™ Transfection Buffer (1x) working solution, the white precipitates will disappear. Always keep PepMute™ Transfection Buffer working solution (1x) at RT.

**Step II. Cell Seeding:**
Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 60-70% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30-60 min before transfection.

**Note:** PepMute™ reagent is NOT interfered by serum and antibiotics, therefore serum and antibiotic containing medium can be used during the entire experiment.

**Table 2. A Guideline for DNA & siRNA Co-transfection Per Cell Culture Vessel**

<table>
<thead>
<tr>
<th>Culture Dish</th>
<th>Growth Medium (mL)</th>
<th>Transfection Buffer (µL)</th>
<th>Plasmid DNA (µg)</th>
<th>siRNA (pmoles) Final 5.0 nM</th>
<th>PepMute™ Reagent (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-well</td>
<td>0.5</td>
<td>50</td>
<td>0.25</td>
<td>2.5</td>
<td>1.5</td>
</tr>
<tr>
<td>12-well</td>
<td>0.75</td>
<td>75</td>
<td>0.375</td>
<td>3.25</td>
<td>2.25</td>
</tr>
<tr>
<td>6-well</td>
<td>1.0</td>
<td>100</td>
<td>0.5</td>
<td>5.0</td>
<td>3</td>
</tr>
<tr>
<td>60 mm</td>
<td>3.0</td>
<td>300</td>
<td>1.5</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>10 cm / flask 75</td>
<td>8.0</td>
<td>800</td>
<td>4.0</td>
<td>40</td>
<td>24</td>
</tr>
</tbody>
</table>

**Step III. DNA & siRNA co-transfection protocol:**
For DNA/siRNA co-transfection experiment, we recommend using 0.5-0.6 µg DNA and 1-20 nM siRNA per well in a 6-well plate. As a starting point, we recommend using 0.5 µg DNA and 5.0 pmoles siRNA (final concentration 5.0 nM) per well of a 6-well plate which usually give satisfactory knockdown effect.

The following conditions are given per well of a 6 well plate. For other culture format, please refer to **Table 2**.
- For each well, add 1.0 mL of complete medium with serum and antibiotics freshly 30-60 min before transfection.
- Dilute 0.5 µg DNA and 5.0 pmoles siRNA (final 5.0 nM) into 100 µL of working solution of PepMute™ Transfection Buffer. Vortex briefly to mix.

**Note:** For optimal transfection efficiency and maximum gene silencing, PepMute™ Transfection Buffer (1x) is a must for diluting siRNA/DNA and PepMute™ reagent.

We strongly suggest preparing siRNA stock solution at 10 µM, so add 0.5 µL siRNA stock solution per well of 6-well plate to make final 5.0 nM of siRNA.
- Add 3 µL PepMute™ reagent immediately, mix by pipetting up and down.
- Incubate for ~15 min at RT to let transfection complex form.

**Note:** Never keep the complex longer than 30 min.
- Add the transfection complex to the cells drop wise.

**Storage:** PepMute™ siRNA Transfection Reagent is stable for up to 12 months at 4°C. Always keep PepMute™ Transfection Buffer (5x) at RT. This item is shipped at ambient temperature.