Supplied in: 10 mM Tris-HCl (pH 8.0), 1 mM EDTA. Dividing in several aliquots is recommended to avoid multiple freeze-thaw cycles.

**Preparation:** The 9 proprietary plasmids are purified, phenol extracted and equilibrated to 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA.

**Reagents Supplied:**
- 6X Gel Loading Dye, Blue
- 1X Gel Loading Dye, Blue:
  - 2.5% Ficoll-400
  - 3.3 mM Tris-HCl (pH 8.0@25°C)
  - 0.017% SDS
  - 0.015% bromophenol blue

**Notes On Use:** This ladder may contain some traces of nicked DNA and dimers above the 10 kb plasmid. To minimize nicking of the supercoiled DNA, always use sterile pipette tips and avoid multiple freeze-thaw cycles. The migration of supercoiled plasmids in agarose gels can change depending on agarose concentration, buffer and electrophoresis conditions. Dilute in TE or other buffer of minimal ionic strength. DNA may denature if diluted in dH2O.

The following protocol is recommended for a 5 mm wide lane.

1. **Prepare loading mixture:**
   - Distilled water
   - 6X Blue Loading Dye
   - DNA Ladder
   - Total volume

2. Mix gently

3. Load onto the agarose gel (See other side)

**Usage Recommendation:** Centrifuge briefly and mix gently before use. We recommend loading 0.5 µg (1 µl) of the Supercoiled DNA Ladder diluted in sample buffer. This ladder was not designed for precise quantification of DNA mass, but can be used for approximating the mass of DNA in comparably intense samples of similar size. The approximate mass of DNA in each of the bands in our Supercoiled DNA ladder is as follows (assuming a 0.5 µg loading):

<table>
<thead>
<tr>
<th>Band</th>
<th>Base Pairs</th>
<th>DNA Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10,000</td>
<td>45 ng</td>
</tr>
<tr>
<td>2</td>
<td>8,000</td>
<td>45 ng</td>
</tr>
<tr>
<td>3</td>
<td>6,000</td>
<td>45 ng</td>
</tr>
<tr>
<td>4</td>
<td>5,000</td>
<td>136 ng</td>
</tr>
<tr>
<td>5</td>
<td>4,000</td>
<td>45 ng</td>
</tr>
<tr>
<td>6</td>
<td>3,500</td>
<td>45 ng</td>
</tr>
<tr>
<td>7</td>
<td>3,000</td>
<td>45 ng</td>
</tr>
<tr>
<td>8</td>
<td>2,500</td>
<td>45 ng</td>
</tr>
<tr>
<td>9</td>
<td>2,017</td>
<td>45 ng</td>
</tr>
</tbody>
</table>

Suggested Protocol for Loading a Sample:

The following protocol is recommended for a 5 mm wide lane.

1. Prepare loading mixture:
   - Distilled water
   - 6X Blue Loading Dye
   - DNA Ladder
   - Total volume

2. Mix gently

3. Load onto the agarose gel (See other side)
Note: The components of the mixture should be scaled up or down, depending on the width of the agarose gel.

Reference: