Supplied in: 10 mM Tris-Cl (pH 8.0), 1 mM EDTA

Reagents supplied:
6X Gel Loading Dye, Blue

1X Gel Loading Dye, Blue:
2.5% Ficoll-400
11 mM EDTA
3.3 mM Tris-Cl (pH 8.0@25°C)
0.017% SDS
0.015% bromophenol blue

Preparation: The double-stranded DNA is digested to completion with appropriate restriction enzymes, phenol extracted and equilibrated to 10 mM Tris-Cl (pH 8.0) and 1 mM EDTA.

Usage Recommendation: We recommend loading 0.5 µg of 100 bp DNA Ladder diluted in sample buffer. The 100 bp DNA 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 100 bp DNA Ladder is as follows (assuming a 0.5 µg loading):

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Base Pairs</th>
<th>DNA Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,517</td>
<td>45 ng</td>
</tr>
<tr>
<td>2</td>
<td>1,200</td>
<td>35 ng</td>
</tr>
<tr>
<td>3</td>
<td>1,000</td>
<td>95 ng</td>
</tr>
<tr>
<td>4</td>
<td>900</td>
<td>27 ng</td>
</tr>
<tr>
<td>5</td>
<td>800</td>
<td>24 ng</td>
</tr>
<tr>
<td>6</td>
<td>700</td>
<td>21 ng</td>
</tr>
<tr>
<td>7</td>
<td>600</td>
<td>18 ng</td>
</tr>
<tr>
<td>8</td>
<td>500,517</td>
<td>97 ng</td>
</tr>
<tr>
<td>9</td>
<td>400</td>
<td>38 ng</td>
</tr>
<tr>
<td>10</td>
<td>300</td>
<td>29 ng</td>
</tr>
<tr>
<td>11</td>
<td>200</td>
<td>25 ng</td>
</tr>
<tr>
<td>12</td>
<td>100</td>
<td>48 ng</td>
</tr>
</tbody>
</table>

Notes: All fragments have 4-base, 5’ overhangs that can be end labeled using T4 Polynucleotide Kinase (NEB #M0201) or filled-in using DNA Polymerase I, Klenow Fragment (NEB #M0210) (1). Use α-[32P] dATP or α-[32P] dTTP for the fill-in reaction.

100 bp DNA Ladder is stable for at least 3 months at 4°C.

For long term storage store at –20°C. If samples need to be diluted, use TE or other buffer of minimal ionic strength. DNA may denature if diluted in dH₂O.
Due to the limitations of the acrylamide gel technology, one or two extra bands may be visible on the DNA ladders when run on a polyacrylamide gel.

**Suggested protocol for loading a sample:**
The following protocol is recommended for a 5 mm wide lane.

1. Prepare loading mixture:
   - Distilled water 4 µl
   - 6X Blue Loading Dye 1 µl
   - DNA Ladder 1 µl
   - Total volume 6 µl
2. Mix gently
3. Load onto the agarose gel

**Note:** The components of the mixture should be scaled up or down, depending on the width of the agarose gel.

Reference: