**PCR Marker**

**N3234S**

100 gel lanes (30 µg)  Lot: 0071308  Exp: 8/15
300 µg/ml  Store at –20°C (see note)
1.5 ml Gel Loading Dye, Blue (6X)  Store at 25°C

**Description:** The PCR Marker consists of a proprietary plasmid that is digested to completion with appropriate restriction enzymes to yield 5 double-stranded DNA bands that are suitable for use as molecular weight standards for agarose and acrylamide gel electrophoresis. The digested DNA includes fragments ranging from 50–766 base pairs.

Supplied in: 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
11 mM EDTA
3.3 mM Tris-HCl (pH 8.0@25°C)
0.017% SDS
0.015% bromophenol blue

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

**Usage Recommendation:** We recommend loading 0.3 µg of PCR Marker diluted in sample buffer. This marker 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 PCR Marker is as follows (assuming a 0.3 µg loading):

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Base Pairs</th>
<th>DNA Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>766</td>
<td>62 ng</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>40 ng</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>48 ng</td>
</tr>
<tr>
<td>4</td>
<td>150</td>
<td>61 ng</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>89 ng</td>
</tr>
</tbody>
</table>

**Notes:** PCR Marker 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.

All ends have 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] dCTP or α-[³²P] dGTP for the fill-in reaction.

**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.

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