Supercoiled DNA Ladder

NEW ENGLAND BioLabs Inc.
1-800-632-7799
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www.neb.com

N0472S 001120414041

N0472S

100 gel lanes (50 µg) **Lot: 0011204 Exp: 4/14**

500 µg/ml **Store at –20°C**

1.5 ml Gel Loading Dye, Blue (6X) **Store at 25°C**

Description: The Supercoiled DNA ladder contains 9 proprietary supercoiled plasmids, ranging in size from 2 to 10 kb, that are suitable for use as supercoiled molecular weight standards for agarose electrophoresis. The 5 kb plasmid has an increased intensity to serve as a reference band.

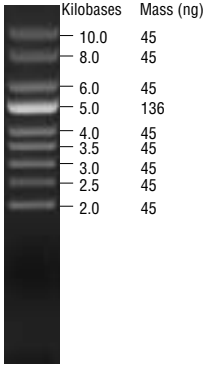
Supplied in: 10 mM Tris-HCl (pH 8.0), 1 mM EDTA. Dividing in several aliquots is recommended to avoid multiple freeze-thaw cycles.

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
11 mM EDTA
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 dH_20



0.5 µg of Supercoiled DNA Ladder visualized by ethidium bromide staining on a 0.8% TAE agarose gel.

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):

Band	Base Pairs	DNA Mass
1	10,000	45 ng
2	8,000	45 ng
3	6,000	45 ng
4	**5,000**	**136 ng**
5	4,000	45 ng
6	3,500	45 ng
7	3,000	45 ng
8	2,500	45 ng
9	2,017	45 ng

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

(See other side)

CERTIFICATE OF ANALYSIS

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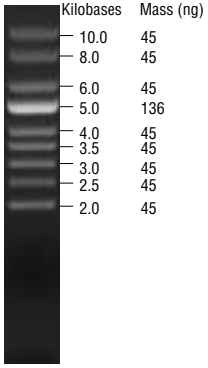

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Note: The components of the mixture should be scaled up or down, depending on the width of the agarose gel.

Reference:

1. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning*: *A Laboratory Manual*, (2nd ed.), Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

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