100 bp DNA Ladder

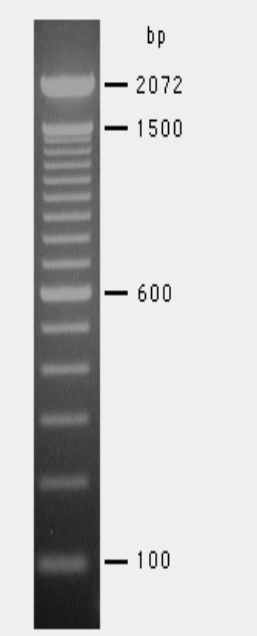
Cat. No. 15628-050 **Size: 250 µg**
Conc.: 1 µg/µl **Store at -20°C.**

Description:
The 100 bp DNA Ladder consists of 15 blunt-ended fragments between 100 and 1500 bp in multiples of 100 bp and an additional fragment at 2072 bp. The 600 bp band is approximately 2 to 3 times brighter than the other ladder bands to provide internal orientation. This ladder is not designed for quantitation.

Storage Buffer:
10 mM Tris-HC1 (pH 7.5)
1 mM EDTA

Recommended Procedure:
Add approximately 0.1 µg of ladder per mm lane width. **Do not heat** before loading. We recommend adding NaCl to a final concentration of 20 mM for gel electrophoresis.

Quality Control:
Agarose gel analysis shows that the bands between 100 to 1500 bp are distinguishable. The 600 bp band must be more intense than any other band except the band at 2072 bp.



100 bp DNA Ladder
0.5 µg/lane
2% agarose gel stained with ethidium bromide.

Note:
During 2% agarose gel electrophoresis with tris-acetate (pH 7.6) as the running buffer, bromophenol blue migrates near the 100 bp fragment. The 100 bp band migrates behind the bromophenol blue marker on 6% polyacrylamide gels with tris-borate (pH 8.0) as the running buffer.

Part of the 600 bp band may migrate anomalously slowly in polyacrylamide gels (1,2,3). This band may appear as an extra band near or on top of the 700 bp band.

References:
1. Hsieh, C., et al. (1991) *Mol. Gen. Genet. 225*, 25.
2. Stellwagen, N.C. (1983) *Biochemistry 22*, 6186.
3. Jordan, H. and Hartley, J. (1997) *Focus® 19*, 9.

Labeling Protocols:
To radiolabel the 100 bp DNA Ladder, use T4 DNA polymerase or T4 polynucleotide kinase. T4 DNA polymerase provides higher specific activity with less ^{32}P input. The ladder may contain oligoribonucleotides that are invisible by ethidium bromide staining, but may be labeled by the T4 polynucleotide kinase exchange reaction.

T4 DNA Polymerase Labeling Protocol

Exonuclease Reaction: Degradation of DNA from both 3′ ends.

1. To a 1.5-ml microcentrifuge tube on ice, add the following:

5× T4 DNA polymerase reaction buffer*	4 µl
100 bp DNA Ladder	10 µg
T4 DNA polymerase	40 units
Autoclaved water	to 20 µl

*165 mM Tris acetate (pH 7.9), 330 mM sodium acetate, 50 mM magnesium acetate, 2.5 mM DTT, 500 µg/ml BSA

2. Make sure all components are at the bottom of the tube. Mix gently, but thoroughly. Centrifuge briefly.
3. Incubate 2 min in a 25°C water bath. Cool reaction on ice.

Resynthesis Reaction (Fill-in): This reaction will resynthesize the degraded DNA strands and yield specific activities of 0.5-2 × 10^6 cpm/µg.

4. Take the reaction from Step 3, above, and add the following (keep on ice):

Autoclaved water	8 µl
5× T4 DNA polymerase reaction buffer	6 µl
dATP (2 mM)	5 µl
dGTP (2 mM)	5 µl
dTTP (2 mM).	5 µl
[α -^{32}P]dCTP (3000 Ci/mmol; 10 mCi/ml)	1 µl

5. Mix thoroughly. Centrifuge briefly. Incubate 2 min at 37°C, then add 5 µl of 2 mM dCTP.
6. Incubate 2 min at 37°C. Stop reaction by adding 2.5 µl of 0.5 M EDTA. Centrifuge for 10 s.
7. The cpm incorporated is determined by adding 1 µl of reaction to 24 µl of 250 mM NaCl, 25 mM EDTA. Spot 5 µl of dilution on a glass fiber filter. Place filter in 10% (w/v) TCA + 1% (w/v) pyrophosphate. Wash filter 3 times with 5% (w/v) TCA and then 2 times with ethanol. Dry filter and then count using an appropriate scintillant.
8. Add 5 µl 0.1% (w/v) bromophenol blue, 0.1 mM EDTA, 50% (v/v) glycerol to the sample.
9. Load 1 × 10^5 cpm per lane.

5' DNA Terminus Labeling Protocol (Phosphate Exchange Reaction)

This reaction will yield specific activities of approximately 250,000 cpm/µg.

1. To a 0.5 ml microcentrifuge tube add the following components in order:

Autoclaved water	11 µl
100 bp DNA Ladder (5 µg)	5 µl
5× Exchange Reaction Buffer*	5 µl
[γ-^{32}P]ATP (10 µCi/µl)	3 µl
T4 polynucleotide kinase* (5 or 10 U/µl)	1 µl

*For ordering purposes:
T4 Polynucleotide Kinase Exchange Reaction Buffer: 10456-010 [250 mM imidazole (pH 6.4), 1.5 mM ADP, 60 mM $MgCl_2$, 75 mM 2-mercaptoethanol]
T4 Polynucleotide Kinase: 18004-010, 18004-028

2. Incubate the reaction mixture at 37°C for 30 min. Increasing reaction times beyond 30 min will not increase labeling of the DNA.
3. Stop the reaction by adding 1 µl of 0.5 M EDTA. Centrifuge for 10 s.
4. Determine radioactive incorporation as above.
5. Add 5 µl 0.1% (w/v) bromophenol blue, 0.1 mM EDTA, 50% (v/v) glycerol to the sample.
6. Load 1 × 10^5 cpm per lane.

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