Certificate of Analysis

100bp DNA Ladder:

Part No.	Size
G210A	250µl

Description: The 100bp DNA Ladder is ideal for determining the size of double-stranded DNA from 100–1,500 base pairs. The ladder consists of 11 double-stranded DNA fragments with sizes of 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000 and 1,500bp. The 500bp band is present at triple the intensity of the other fragments and serves as a reference indicator. All other fragments appear with equal intensity on the gel. Recommended loading volume is 5µl/lane.

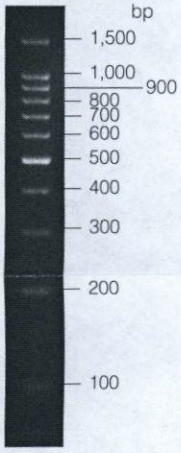
Storage Buffer: The 100bp DNA Ladder is supplied in 10mM Tris-HCl, 1mM EDTA. Final pH 7.4.

Concentration: Five microliters (650ng) of the ladder contains approximately 150ng of the 500bp DNA fragment and 50ng of each of the other ten DNA fragments.

Storage Conditions: Store at –20°C.

Usage Note: Concentration gradients may form in frozen products and should be mixed well prior to use.

Blue/Orange 6X Loading Dye (G190A): The Blue/Orange 6X Loading Dye supplied with these markers has a composition of 15% Ficoll® 400, 0.03% bromophenol blue, 0.03% xylene cyanol FF, 0.4% orange G, 10mM Tris-HCl (pH 7.5) and 50mM EDTA. This dye is used for loading DNA samples into gel electrophoresis wells and tracking migration during electrophoresis. Recommended usage is one part loading dye for every five parts DNA solution. The xylene cyanol FF migrates at approximately 4kb, bromophenol blue at approximately 300bp and orange G at approximately 50bp in 0.5% to 1.4% agarose gels in 0.5X TBE (1).



2% agarose

Quality Control Assays

Accurate Sizing: Five microliters of the 100bp DNA Ladder are mixed with 1µl of Loading Dye and subjected to electrophoresis on a 2% agarose gel with TAE 1X buffer. The markers must show the expected pattern when compared with HaeIII-digested ΦX174 DNA Markers (Cat.# G1761).

Nuclease Assay: To test for nuclease contamination, 5µl of the 100bp DNA Ladder are incubated in restriction enzyme buffer overnight at 37°C. Following incubation, the ladder is subjected to electrophoresis and visualized on an ethidium bromide-stained agarose gel to verify the absence of visible degradation.

5 End-Labeling: Five microliters of the 100bp DNA Ladder are added to a labeling reaction containing 1µl of T4 Polynucleotide Kinase 10X Buffer, 1µl of [γ-^{32}P]ATP (3,000Ci/mmol @ 10µCi/µl), 1µl of T4 Polynucleotide Kinase and 2µl of deionized water. This reaction is incubated at 37°C for 10 minutes, then stopped by the addition of 1µl of 0.5M EDTA. After labeling, the 100bp DNA Ladder is separated on a 4% nondenaturing polyacrylamide gel. After the gel is processed, the labeled markers must be easily visible after overnight exposure to X-ray film without an intensifying screen at –70°C.

Reference

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

Signed by:

R. Wheeler, Quality Assurance

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