

1 Kb Plus DNA Ladder

Cat. No. 10787-026

Conc.: 1 µg/µl

Size: 1000 µg

Store at -20°C.

Description:

The 1 Kb Plus DNA Ladder is suitable for sizing linear double-stranded DNA fragments from 100 bp to 12 kb. The ladder contains a total of twenty bands: twelve bands ranging in size from 1000 bp to 12,000 bp in 1000-bp increments and eight bands ranging in size from 100 to 1650 bp. The 1650-bp band contains approximately 8% of the mass applied to the gel. The ladder may be radioactively labeled by one of the following methods: (i) Partial exonucleolytic degradation and resynthesis with T4 DNA polymerase. This method is preferred because higher specific activity is achieved with less ³²P input; (ii) Labeling the 5' ends with T4 polynucleotide kinase; (iii) Filling in the 3' recessed ends with *E. coli* DNA polymerase I or the large fragment of DNA polymerase I.

Storage Buffer:

10 mM Tris-HCl (pH 7.5)

1 mM EDTA

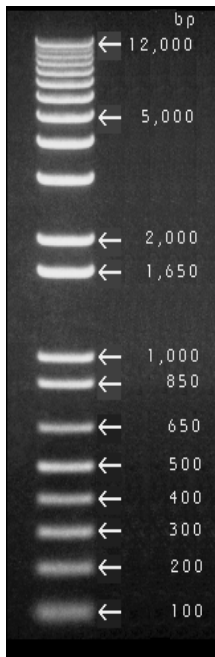
50 mM NaCl

Recommended Procedure:

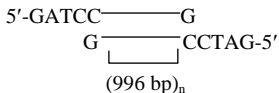
Invitrogen recommends the use of 10X BlueJuice™ Gel Loading Buffer (Cat. No. 10816-015) at a concentration of 2X for electrophoresis of DNA standards on agarose gels. Alternately, the DNA standard can be diluted such that the final concentration of NaCl is 20 mM. Apply approximately 0.1 µg of ladder per mm lane width. **Do not heat** before loading.

Quality Control:

Agarose gel analysis shows that all bands in the ladder are distinguishable and are of approximate equal intensity by ethidium bromide staining.



Structure of Fragments in 1-Kb Increments:



Notes:

During 1% agarose gel electrophoresis with Tris-acetate (pH 7.5) as the running buffer, bromophenol blue migrates together with the 500 bp band.

The 1650 bp band is generated from pUC. The bands smaller than 1000 bp are derived from lambda DNA.

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0.7 µg/lane

0.9% agarose gel

stained with ethidium bromide

Labeling Protocols:***T4 DNA Polymerase Labeling Protocol***

1. Exonuclease Reaction (Degradation of DNA from both 3'-ends)
 - a. To a 1.5-ml microcentrifuge tube on ice, add the following:

5X T4 DNA polymerase reaction buffer [165 mM Tris acetate (pH 7.9), 330 mM sodium acetate, 50 mM magnesium acetate, 2.5 mM DTT, 500 µg/ml BSA].....	4 µl
1 Kb Plus DNA Ladder	10 µg
T4 DNA polymerase.....	40 units
Autoclaved water.....	to 20 µl
 - b. Make sure all components are at the bottom of the tube. Mix thoroughly but not vigorously. Centrifuge briefly.
 - c. Incubate 2 min at 37°C. (about 25 nucleotides/min are removed). Cool reaction vial on ice.
2. Resynthesis Reaction (Fill-in)

This reaction will resynthesize the degraded DNA strands.

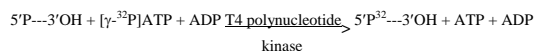
 - a. Place into the reaction vial which is sitting in ice after the exonuclease reaction:

Autoclaved water	8 µl
5X T4 DNA polymerase reaction buffer.....	6 µl
dCTP (2 mM).....	5 µl
dGTP (2 mM)	5 µl
dTTP (2 mM).....	5 µl
[α - ³² P]dATP (3000 Ci/mmol; 10 mCi/ml).....	1 µl
 - b. Mix thoroughly. Centrifuge briefly. Incubate 2 min at 37°C, then add 5 µl of 2 mM dATP.
 - c. Incubate 2 min at 37°C. Stop reaction by adding 2.5 µl of 0.5 M EDTA. Centrifuge for 10 s.
 - d. 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. The filter is dried and then counted using an appropriate scintillant.

- e. Add 5 µl 0.1% (w/v) bromophenol blue, 0.1 mM EDTA, 50% (v/v) glycerol to the sample.
- f. Load 1×10^5 cpm in a lane.

5' DNA Terminus Labeling Protocol (Phosphate Exchange Reaction)

This reaction will yield specific activities of approximately $1-5 \times 10^5$ cpm/pmol of ends.



1. Add the following components to a 0.5-ml microcentrifuge tube in the following order:
Autoclaved water.....11 µl
1 Kb Plus DNA Ladder (5 µg).....5 µl
*5X exchange reaction buffer [250 mM imidazole (pH 6.4), 1.5 mM ADP, 60 mM MgCl₂, 75 mM 2-mercaptoethanol].....5 µl
[γ-³²P]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
T4 Polynucleotide Kinase: 18004-010, 18004-028
2. Incubate the reaction mixture at 37°C for 30 minutes. Increasing reaction times beyond 30 min will not increase labeling of the DNA.
3. Stop 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% (w/v) glycerol to the sample.
6. Load 1×10^5 cpm in a lane.

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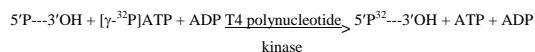
Part no. 10787026.pps

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