Invitrogen
life technologies

1 Kb DNA Extension Ladder

Cat. No. 10511-012 **Size: 100 µg**

Conc. 1 µg/µl **Store at -20°C**

Description

The 1 Kb DNA Extension Ladder is suitable for sizing linear double-stranded DNA fragments from 500 bp to 40 kb. This ladder consists of bands containing one to eight repeats of a 1018-bp DNA fragment; vector bands of 506 bp, 517 bp, and 1636 bp; and bands of 5 kb, 10 kb, 20 kb, and 40 kb. The doublet at 1.6 and 2 kb, the gap between 8 and 10 kb, and the extra bright 5 kb band allow easy orientation within the ladder. Vector bands smaller than 500 bp are also present but may not be visible or resolved. The 1 Kb DNA Extension Ladder may be labeled by one of the following methods: (a) partial exonucleolytic degradation and resynthesis with T4 DNA polymerase, (b) labeling the 5′ ends with T4 polynucleotide kinase; (c) filling in the 3′ recessed ends with *E. coli* DNA polymerase I or the large fragment of DNA polymerase I. All bands are labeled with the fill-in method using labeled dATP, provided the other three dNTPs are included.

Storage Buffer

10 mM Tris-HCl (pH 7.5)
5 mM NaCl
0.1 mM EDTA

Recommended Procedure

We recommend a final concentration of 20 mM NaCl in the sample to be loaded on a gel. Apply approximately 0.1 µg of ladder per mm lane width. **Do not heat** before loading. The following electrophoresis conditions will yield useful resolution of the respective DNA fragments (where cm refers to the distance between the electrodes of the electrophoresis apparatus):

10-15 kb, 1% agarose, 2.5 volts/cm, 2-4 h
15-20 kb, 1% agarose, 2.5 volts/cm, 2-4 h
20-40 kb, 0.7% agarose, 1.3 volt/cm, 4 h

Part No. 10511012.pps Rev. Date: 03/20/03

For technical questions about this product, call the Invitrogen Tech-Line℠ U.S.A. 800 955 6288

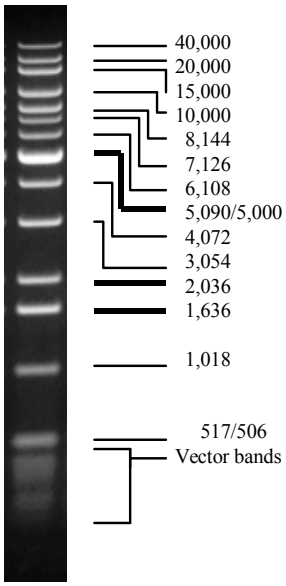

Figure
0.5 µg of 1 Kb DNA Extension Ladder, 0.7% gel, TAE buffer, 0.5 µg/ml ethidium bromide in gel and buffer, a horizontal gel apparatus, 8-tooth 0.8-mm comb, 22 volts (1.3 V/cm), 4 hours.

Quality Control
Agarose gel analysis shows that all bands larger than 500 bp are distinguishable.

Note: If the ionic strength of the sample is too low, blurring of the bands can occur.

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 DNA Extension 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:

      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.

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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 x 10^5 cpm/pmol of ends.

$$5'P\text{---}3'OH + [\gamma\text{-}^{32}P]ATP + ADP \xrightarrow[\text{kinase}]{\text{T4 polynucleotide}} 5'P^{32}\text{---}3'OH + ATP + ADP$$

1. Add the following components to a 0.5-ml microcentrifuge tube in the following order:
   Autoclaved water ..... 11 µl
   1 Kb DNA Extension Ladder (5 µg) ..... 5 µl
   5X exchange reaction buffer
   [250 mM imidazole (pH 6.4), 350 µM ADP,
   60 mM $MgCl_2$, 5 mM 2-mercaptoethanol] ..... 5 µl
   [γ-^{32}P]ATP (10 µCi/µl) ..... 3 µl
   T4 polynucleotide kinase (5 or 10 U/µl) ..... 1 µl
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.

Additional Products

Product Name	Catalog Number	Amount
T4 Polynucleotide Kinase	18004-010	200 U
	18004-028	1,000 U
T4 Polynucleotide Kinase Exchange Reaction Buffer	10456-010	2 × 1 ml