Invitrogen
life technologies

1 Kb DNA Ladder

Cat. No. 15615-016 **Size: 250 µg**
Conc.: 1.0 µg/µl **Store at -20°C.**

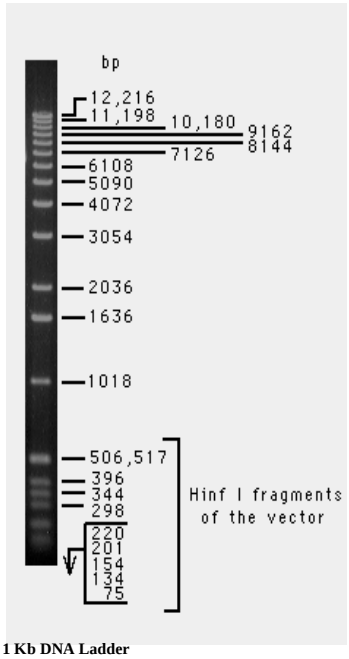
Description:
The 1 Kb DNA Ladder (U.S. Patent No. 4,403,036) is suitable for sizing linear double-stranded DNA fragments from 500 bp to 12 kb. The bands of the ladder each contain from 1 to 12 repeats of a 1018-bp DNA fragment. In addition to these 12 bands, the ladder contains vector DNA fragments that range from 75 to 1636 bp. The 1636-bp band contains 10% 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 ^{32}P 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)
50 mM NaCl
0.1 mM EDTA

Recommended Procedure:
Invitrogen recommends the use of 10X BlueJuice® Gel Loading Buffer (10816-015) at a concentration of 2X [for electrophoresis of this ladder on agarose gels]. Alternatively, the DNA ladder can be diluted in a buffer such that the final concentration of NaCl is 20 mM. Apply approximately 0.1 µg of standard per mm lane width. DO NOT HEAT!

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

Doc. Rev.: 011602



1 Kb DNA Ladder
0.5 µg/lane
0.9% agarose gel
stained with ethidium bromide

Structure of Fragment (1):

5′-TCGGG———C
C———GAGCC-5′
1014 bp

Notes:
During 1.2% agarose gel electrophoresis with Tris-acetate (pH 7.6) as the running buffer, bromophenol blue migrates together with the 506/517 bp doublet band.

The 1636 bp band and all bands less than 1000 bp are generated from pBR322.

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 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 x 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 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

   *For ordering purposes:
   T4 Polynucleotide Kinase Exchange Reaction Buffer: 10456-010
   T4 Polynucleotide Kinase (includes buffer): 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.

Reference:

1. Hartley, J.L. and Gregori, T.J. (1981) *Gene 13*, 347.