Invitrogen
life technologies

50 bp DNA Ladder

Cat. No.: 10416-014 **Size: 50 µg**
Conc.: 1 µg/µl **Store at -20°C.**

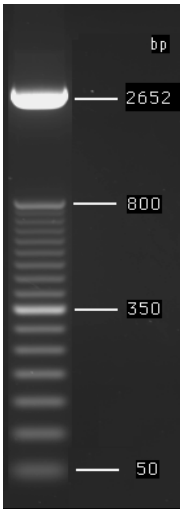
Description:
The 50 bp DNA Ladder consists of 16 blunt-ended fragments between 50 and 800 bp in multiples of 50 bp and an additional fragment at 2652 bp. The 350-bp band is approximately 2 to 3 times brighter than other ladder bands to provide internal orientation. This ladder is not designed for quantitation.

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

Recommended Procedure:
A final concentration of 20 mM NaCl is recommended for gel electrophoresis. Apply approximately 0.1 µg of ladder per mm lane width. **Do not heat** before loading.

Quality Control:
Agarose gel analysis shows that the bands between 50 to 800 bp are distinguishable. The 350-bp orientation band must be distinguishable in intensity.

Doc. Rev.: 110901



Note: The 50 bp DNA Ladder may be suitable for electrophoresis on native 6% polyacrylamide gels (1).

References:

1. Jordan, H. and Hartley, J. (1997) *FOCUS® 19*, 9.

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

Labeling Protocols:

The 50 bp DNA Ladder can be radioactively labeled by T4 DNA polymerase or T4 polynucleotide kinase. T4 DNA polymerase is recommended because higher specific activity is achieved with less ^{32}P input. The ladder may contain oligoribonucleotides which are invisible with ethidium bromide staining, but may be labeled by the T4 polynucleotide kinase exchange reaction.

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 [330 mM sodium acetate, 165 mM Tris acetate (pH 7.9), 50 mM magnesium acetate, 2.5 mM DTT, 500 µg/ml BSA].....4 µl
      50 bp 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 in a 25°C water bath. Cool reaction vial on ice.
2. Resynthesis Reaction (Fill-in)

   This reaction will resynthesize the degraded DNA strands and yield specific activities of $0.5\text{-}2 \times 10^6$ cpm/µg.

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

      Autoclaved water.....8 µl
      5X 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

   b. Mix thoroughly. Centrifuge briefly. Incubate 2 min at 37°C, then add 5 µl of 2 mM dCTP.

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 of 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 250,000 cpm/µg.

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

1. Add the following components to a 1.5-ml microcentrifuge tube in the following order:

   Autoclaved water .....4 µl
   50 bp 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)..... 10 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 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 of 0.1% (w/v) bromophenol blue, 0.1 mM EDTA, 50% (v/v) glycerol to the sample. Load 1×10^5 cpm in a lane.