Invitrogen
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30–330-bp AFLP® DNA Ladder

Cat. No. 10832-012 **Size: 300 applications**

Store at -20°C

Description

The 30–330-bp AFLP® DNA ladder consists of 32 10-bp repeats. Additionally, there are diffuse bands at 10 bp and 20 bp, plus a fragment at 1668 bp. This ladder is specifically designed for sizing AFLP® DNA fragments and can be visualized on a 5–6% urea-polyacrylamide gel following electrophoresis of the end-labeled product.[1,2] Because both DNA strands are the same nucleotide composition, the denatured product produces a set of single-stranded oligonucleotides increasing in length by 10-bp increments. This product is easily radiolabeled using T4 polynucleotide kinase.

Storage Buffer

10 mM Tris-HCl (pH 7.5)
1 mM EDTA

Recommended Protocol:

30–330-bp AFLP® DNA ladder is end-labeled with ^{32}P or ^{33}P with T4 polynucleotide kinase using the exchange reaction in the following protocol. Apply approximately 2 µl of ladder per lane on a 5-6% urea-polyacrylamide gel.

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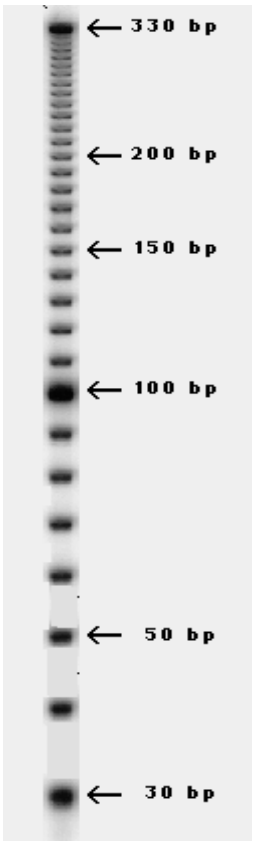

Note: The 100-bp band will appear two to three times brighter than other bands.

30–330-bp AFLP® DNA Ladder
labeled with ^{32}P
2 µl ladder/reaction
6% polyacrylamide/urea gel

Labeling of the 30–330-bp AFLP® DNA ladder with ^{32}P or ^{33}P

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

or

$$5'P\text{---}3'OH + [\gamma\text{-}^{33}P]ATP + ADP \xrightarrow[\text{kinase}]{\text{T4 polynucleotide}} 5'P^{33}\text{---}3'OH + ATP + ADP$$

1. Pipet into a 0.5-ml microcentrifuge tube on ice:
   2 µl 30–330-bp AFLP® DNA ladder
   1 µl 5X Exchange Reaction Buffer [250 mM imidazole (pH 6.4), 60 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 350 µM ADP]
   1 µl $\gamma^{32}P$ ATP (>3,000 Ci/mmole, 10 µCi/µl) or 1 µl $\gamma^{33}P$ ATP (>3,000 Ci/mmole, 10 µCi/µl)
   1 µl T4 polynucleotide kinase (10 U/µl)
2. Make sure all components are at the bottom of tube. Mix thoroughly but not vigorously. Centrifuge briefly.
3. Incubate for 10 min at 37°C.
4. Stop the reaction by heating the tube 15 min at 65°C.
5. This reaction will yield specific activities of approximately 2 x 10^6 cpm/µl.
6. To 5 µl of 30–330-bp AFLP® DNA ladder reaction mix, add an equal volume of TE buffer, pH7.5, and 25 µl denaturing solution [98% (v/v) formamide, 10 mM EDTA (pH 8.0), 0.025% (w/v) bromophenol blue, 0.025% (w/v) xylene cyanol]. Total volume = 35 µl.
7. Incubate at 70°C for 5 min.
8. Electrophorese 2 µl of the sample in a denaturing 5-6% polyacrylamide/urea gel following the same protocol for AFLP® analysis. A set of single-stranded oligonucleotides (30–330 nucleotides) increasing in length of 10 nucleotide increments should be clearly visible after exposured in x-ray film.

Note: Diffuse bands at 10 bp and 20 bp are also visible.

Quality Control

Electrophoresis of the 30–330-bp AFLP® DNA ladder on a 4% Low Melting Point agarose gel shows that the bands between 30 bp to 330 bp are distinguishable. The 100-bp band is two to three times brighter than the rest of the bands.

Additional Products

Product	Catalog no.
T4 Polynucleotide Kinase	18004-010/18004-028
T4 Polynucleotide Kinase Exchange Buffer	10456-010
AFLP® Analysis System I	10544-013
AFLP® Analysis System II	10717-015

References

1. Vos, P., et. al. (1995) *Nucleic Acids Res*. 23, 4407.
2. Lin, J.-J., et. al. (1995) *FOCUS® 17*, 66.