pUC19/Sau3A I Digest dsDNA Markers

Store at –20°C.
Do not store in a frost-free freezer.

Catalog # (P/N):	AM7760
Amount:	50 µg
Concentration:	0.5 mg/mL
Storage Conditions:	Store at –20°C. This product is shipped at ambient temperature. This in no way affects its high-quality performance. Upon receipt, store at –20°C. Avoid multiple freeze-thaw cycles. ***Do not store in a frost-free freezer.*** Aliquots of the product may be stored short-term at 4°C.
Storage Buffer:	10 mM Tris pH 7.5, 1 mM EDTA

USER INFORMATION

Product Description: pUC19/Sau3A I dsDNA Markers provide a set of double-stranded DNA size standards, 955 bp, 585 bp, 341 bp, 258 bp, 141 bp, 105 bp, 78/75 bp, 46 bp, 36 bp, 18/17 bp, 12/11 bp, and 8 bp in length.

Applications: pUC19/Sau3A I dsDNA Markers are intended for use as size markers for gel electrophoresis. They can be visualized after electrophoresis by ethidium bromide staining and UV fluorescence; typically 9 bands will be visible on an agarose gel using 1.0 µg of marker. These markers can be end labeled using Klenow Polymerase or T4 Polynucleotide Kinase for visualization by autoradiography after electrophoresis on a 8% polyacrylamide/8 M urea gel or 5% polyacrylamide gel, using ~10^5 cpm (see Figure 1).

Ethidium Bromide Staining
The markers may be visualized by several methods.

A. Add ethidium bromide to the sample at 10–50 µg/mL final concentration before loading.

B. Incorporate ethidium bromide into the gel ***or*** running buffer at 0.5 µg/mL.

C. Stain post-electrophoresis using 0.5 µg/mL ethidium bromide in gel running buffer (e.g., 1 µL of 10 mg/mL ethidium in 20 mL of buffer).

Note: For methods B or C, destain for 5–10 min in buffer without ethidium bromide.

End Labeling with [α-^{32}P]dNTP and Klenow Polymerase

For a 20 µL end-labeling reaction, use the following reaction conditions.

200 mM HEPES (pH 7.2)

10 mM Na_3Citrate

10 mM $MgCl_2$

1 mM DTT

50 µM each of 3 dNTPs (i.e., either no dATP or no dCTP)

5 µM unlabeled form of the radiolabeled dNTP

10–20 µCi (3000 Ci/mmol) of [α-^{32}P]dNTP, either dATP or dCTP (equivalent to 0.2–0.3 µM)

<0.5 µg dsDNA Marker

3 U Klenow polymerase

1. Incubate at 37°C for 15 min.
2. Add 100 µL TE.
3. Use labeled markers directly by adding an equal volume of Gel Loading Buffer II (P/N AM8546G), heating to 65°C for 10 min, and loading on the gel.

End Labeling with T4 Polynucleotide Kinase (PNK) and [γ-^{32}P]ATP

We recommend using the Ambion® KinaseMax™ Kit (P/N AM1520). The standard protocol calls for dephosphorylation of the DNA, using alkaline phosphatase, and removal of the alkaline phosphatase, using the Phosphatase Removal Reagent provided with the kit, before performing a forward polynucleotide kinase reaction (PNK catalyzes the transfer of the γ phosphate from [γ-^{32}P]ATP to the 5'-OH created by alkaline phosphatase). Alkaline phosphatase can also be removed by phenol extraction followed by ethanol precipitation.

5' end-exchange reactions are possible using the KinaseMax Kit and DNA which has not been dephosphorylated (i.e., contains 5' phosphate). While labeling by 5' end-exchange is 1/5 as efficient as a PNK forward reaction, the specific activity of the probe will be high enough for use on dozens of lanes, and the reaction is quicker and easier.

Use labeled markers directly by adding an equal volume of Gel Loading Buffer II (P/N AM8546G), heating to 65°C for 10 min and loading on the gel.

(Optional) Removal of Unincorporated Radiolabeled Nucleotides from End-labeled Products by Precipitation

1. Add NH_4OAc to a final concentration of 0.5 M.
2. Add 2.5 volumes of ethanol.
3. Chill at –20°C for 20 min and centrifuge for 10 min.
4. Carefully decant and discard supernatant using appropriate disposal techniques.
5. Add 100 µL of TE and 100 µL of Gel Loading Buffer II.
6. Heat aliquots to 65°C for 10 min before loading.

Visualization by Autoradiography

An aliquot of 1 x 10^5 cpm of ^{32}P-labeled marker (generally 1–2 µL) typically generates bands visible with a 2 hour autoradiograph. As the ^{32}P decays, larger volumes can be used.

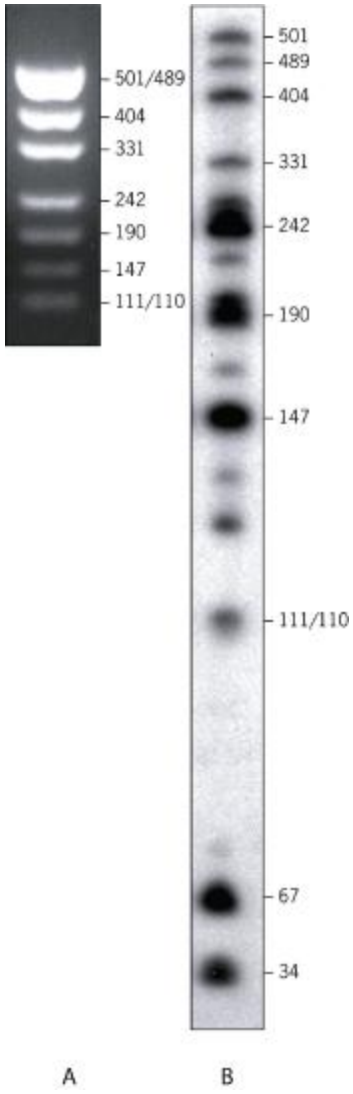

Figure 1. A. 0.5 µg of pUC19/Sau3A I dsDNA Markers was run on a 2% agarose gel until the bromophenol blue dye was 3/4 down the gel, then stained with ethidium bromide. B and C. End-labeled markers were run on a 5% polyacrylamide/8 M urea gel until the bromophenol blue was just off the gel (A) or 3/4 down the gel (B), then visualized by autoradiography.

QUALITY CONTROL

Functional Testing: 1 µg of product is run on an agarose gel with ethidium bromide staining, and the six largest bands are detected. 1 µg of marker is end-labeled, separated by electrophoresis on an 8% polyacrylamide/8 M urea gel, and visualized by autoradiography.

OTHER INFORMATION

Material Safety Data Sheets: Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address: www.ambion.com/techlib/msds. Alternatively, e-mail your request to MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery. For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

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