QIAprep® Spin Miniprep Kit

The QIAprep Spin Miniprep Kit (cat. nos. 27104 and 27106) can be stored at room temperature (15–25°C) for up to 12 months.

For more information, please refer to the *QIAprep Miniprep Handbook*, which can be found at www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

Notes before starting

- **Optional**: Add LyseBlue® reagent to Buffer P1 at a ratio of 1 to 1000. Add the provided RNase A solution to Buffer P1, mix, and store at 2–8°C.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifugation steps are carried out at 13,000 rpm (~17,900 x g) in a conventional table-top microcentrifuge.
- Symbols: ❁ centrifuge processing; ▲ vacuum processing.

1. Pellet 1–5 ml bacterial overnight culture by centrifugation at >8000 rpm (6800 x g) for 3 min at room temperature (15–25°C).
2. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.
3. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times until the solution becomes clear. Do not allow the lysis reaction to proceed for more than 5 min. If using LyseBlue reagent, the solution will turn blue.
4. Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times. If using LyseBlue reagent, the solution will turn colorless.
5. Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.
6. Apply the supernatant from step 5 to the QIAprep spin column by decanting or pipetting. ❁ Centrifuge for 30–60 s and discard the flow-through, or ▲ apply vacuum to the manifold to draw the solution through the QIAprep spin column and switch off the vacuum source.
7. Recommended: Wash the QIAprep spin column by adding 500 µl Buffer PB. ❁ Centrifuge for 30–60 s and discard the flow-through, or ▲ apply vacuum to the manifold to draw the solution through the QIAprep spin column and switch off the vacuum source.

   Note: This step is only required when using *endA*$^+$ strains or other bacterial strains with high nuclease activity or carbohydrate content.
8. Wash the QIAprep spin column by adding 750 µl Buffer PE. ❁ Centrifuge for 30–60 s and discard the flow-through, or ▲ apply vacuum to the manifold to draw the solution through the QIAprep spin column and switch off the vacuum source. Transfer the QIAprep spin column to the collection tube.
9. Centrifuge for 1 min to remove residual wash buffer.
10. Place the QIAprep column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of the QIAprep spin column, let it stand for 1 min, and centrifuge for 1 min.

Product Contents

1kb DNA Ladder:

Part No.	Size
G571A	500µl

Description: The 1kb DNA Ladder is ideal for determining the size of double-stranded DNA from 250–10,000 base pairs. The ladder consists of 13 double-stranded, blunt-end fragments with sizes of 250/253, 500, 750, 1,000, 1,500, 2,000, 2,500, 3,000, 4,000, 5,000, 6,000, 8,000 and 10,000 base pairs. The 1,000 and 3,000bp fragments have increased intensity relative to the other bands on ethidium bromide-stained agarose gels and serve as reference indicators. All other fragments appear with equal intensity on the gel. All fragments are dephosphorylated by CIAP treatment. However, they are not intended for use in quantitative analysis. Recommended loading volume is 5µl/lane.

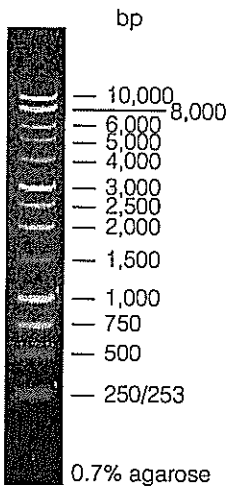
Storage Buffer: The 1kb DNA Ladder is supplied in 10mM Tris-HCl (pH 7.4), 1mM EDTA.

Concentration: The concentration of the 1kb DNA Ladder is 100µg/ml.

Storage Conditions: Store at –20°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. See the expiration date on the product label.

Usage Note: Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

Blue/Orange 6X Loading Dye (G190A): The Blue/Orange 6X Loading Dye supplied with these markers has a composition of 15% Ficoll® 400, 0.03% bromophenol blue, 0.03% xylene cyanol FF, 0.4% orange G, 10mM Tris-HCl (pH 7.5) and 50mM EDTA. This dye is used for loading DNA samples into gel electrophoresis wells and tracking migration during electrophoresis. Recommended usage is one part loading dye for every five parts DNA solution. The xylene cyanol FF migrates at approximately 4kb, bromophenol blue at approximately 300bp and orange G at approximately 50bp in 0.5% to 1.4% agarose gels in 0.5X TBE (1).



Quality Control Assays

Accurate Sizing: Five microliters (500ng) of the 1kb DNA Ladder are mixed with 1µl of Blue/Orange 6X Loading Dye and subjected to electrophoresis in a single lane on a 1.2% agarose gel with TAE 1X buffer. The markers must show the expected pattern when compared with Lambda DNA/*Hin*d III Markers (Cat.# G1711) and ΦX174 DNA/*Hae* III Markers (Cat.# G1761). **Note:** In a 2% gel, the 250bp fragment may appear as a doublet of 253 and 250bp, respectively.

Nuclease Assay: To test for nuclease contamination, 5µl of the 1kb DNA Ladder are incubated in restriction enzyme buffer overnight at 37°C. Following incubation, the ladder is subjected to electrophoresis and visualized on an ethidium bromide-stained agarose gel to verify the absence of visible degradation.

5′ End-Labeling: Five microliters of the 1kb DNA Ladder are added to a labeling reaction containing 1µl of T4 Polynucleotide Kinase 10X Buffer, 1µl of [γ-^{32}P]ATP (3,000Ci/mmol @ 10µCi/µl), 1µl of T4 Polynucleotide Kinase and 2µl of deionized water. This reaction is incubated at 37°C for 10 minutes, then stopped by the addition of 1µl of 0.5M EDTA. **Do not heat-inactivate the reaction. Do not heat the marker before loading.** After labeling, the 1kb DNA Ladder is separated on a 1.2% agarose gel. After the gel is processed, the labeled markers must be easily visible after overnight exposure to X-ray film without an intensifying screen, at room temperature.