006 - QIAprep Spin Miniprep Kit (50)

For 50 high-purity plasmid minipreps: 50 QIAprep 2.0 Spin Columns, Reagents, Buffers, Collection Tubes (2 ml)

This protocol describes the procedure to generate small quantities of pure plasmid DNA using QIAprep Spin Miniprep Kit (Qiagen, Cat.# 27104). This protocol is based on the QIAprep® Miniprep Handbook (December 20202) available on the Qiagen website. It is recommended to thoroughly read the original Qiagen handbook before proceeding with using the kit.

1. Kits contents

Content	**Amount**
QIAprep 2.0 Spin Columns	50
Buffer P1	20 mL
Buffer P2	20 mL
Buffer N3*	30 mL
Buffer PB*	30 mL
Buffer PE (concentrate)	2 × 6 mL
Buffer EB	15 mL
LyseBlue®	20 µL
Loading dye	110 µL
RNase A**	2 mg
Collection tubes (2 mL)	50
Quick-Start Protocol	1

* Buffers N3 and PB contain chaotropic salts which are irritants and not compatible with disinfecting agents containing bleach. Take appropriate laboratory safety measures and wear gloves when handling.

** Provided as a 10 mg/mL solution.

2. Important safety notes

2.1. Always be aware of the safety risks posed by the different chemicals and instruments used while preparing the solutions described in this guide.

2.2. Before starting to work with the chemicals listed in this guide, review the safety data sheet provided with each chemical. If a copy of the safety data sheet is not provided with the chemical, check the website of the manufacturer.

2.3. Always wear the appropriate personal protective equipment (PPE) when handling the chemicals and executing the procedures described in this guide. Examples of PPE that should be worn at all times are:

2.3.1. Safety goggles

2.3.2. Disposable rubber gloves

2.3.3. Face mask (if required)

2.3.4. Laboratory coat

2.4. When handling hot containers use appropriate protective equipment and judgement to avoid injuries.

2.5. When handling chemicals use appropriate protective equipment and judgement to avoid:

2.5.1. Direct contact with the skin or mucosal membranes.

2.5.2. Inhaling powders or vapours.

2.6. When using a gas burner, keep flammable substances at a safety distance from the flame. If using ethanol to sanitise equipment, make sure the ethanol has evaporated before bringing the equipment near the flame.

3. List of abbreviations

Abbreviation	**Full form**
g	Gravitational force (see rcf below)

4. Storage

QIAprep Miniprep Kits should be stored dry at room temperature (15–25°C). Under these conditions, if no expiration date is mentioned on the kit label, QIAprep Miniprep Kits can be stored for up to 12 months without showing any reduction in performance and quality. For longer storage, these kits can be kept at 2–8°C. If any precipitate forms in the buffers after storage at 2–8°C, it should be redissolved by warming the buffers to 37°C before use.

After addition of RNase A and optional LyseBlue reagent, Buffer P1 is stable for 6 months when stored at 2–8°C. RNase A stock solution can be stored for 2 years at room temperature.

5. Equipment and Reagents to Be Supplied by User

5.1. Standard microbiological equipment for growing and harvesting bacteria (e.g., inoculating loop, culture tubes and flasks, and 37°C shaking incubator).

5.2. Centrifuge with rotor for 96-well blocks.

5.3. 96–100% ethanol

5.4. 10 µL mechanical pipette and tips

5.5. 20 µL mechanical pipette and tips

5.6. 200 µL mechanical pipette and tips

5.7. 1000 µL mechanical pipette and tips

5.8. Sharpie marker

6. Things to do before starting

6.1. Add the provided RNase A solution to Buffer P1 before use. Use 1 vial RNase A (centrifuge briefly before use) per bottle Buffer P1 for a final concentration of 100 µg/mL. Mix and store at 2–8°C.

6.2. Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).

6.3. Check Buffers P2 and N3 before use for salt precipitation. Redissolve any precipitate by warming to 37°C. Do not shake Buffer P2 vigorously.

6.4. Close the bottle containing Buffer P2 immediately after use to avoid acidification of Buffer P2 from CO2 in the air.

6.5. Buffers P2, N3 and PB contain irritants. Wear gloves when handling these buffers.

6.6. Add the provided LyseBlue reagent to Buffer P1 and mix before use. Use 1 vial LyseBlue reagent per bottle Buffer P1 for a final dilution of 1:1000 (e.g., 10 µl LyseBlue into 10 ml Buffer P1). LyseBlue provides visual identification of optimum buffer mixing, thereby preventing the common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris.

7. Procedure

7.1. Preparation of the bacterial culture

7.1.1. Pick a single colony from a freshly streaked selective plate and inoculate a culture of 5 ml LB medium containing the appropriate selective antibiotic. Incubate for 12–16 h at 37°C with vigorous shaking.

Growth for more than 16 h is not recommended because cells begin to lyse and plasmid DNA yields may be reduced. Use a tube or flask with a volume of at least 4 times the volume of the culture.

7.1.2. Harvest 1.5 mL of the bacterial cells by centrifugation at 6800 × *g* in a conventional table-top microcentrifuge for 3 min at room temperature.

7.1.3. Discard the supernatant and proceed to step 7.2. Alternatively, the bacterial pellets can be stored at -20˚C until further processing.

7.2. Plasmid isolation (miniprep)

7.2.1. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.

Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.

If LyseBlue reagent has been added to Buffer P1, before use vigorously shake the buffer bottle to ensure LyseBlue particles are completely dissolved. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

7.2.2. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times.

Mix gently by inverting the tube. Do not vortex, because this will result in shearing of genomic DNA and contamination of plasmid. Continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

If LyseBlue has been added to Buffer P1, the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localised colourless regions, or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

7.2.3. Add 350 µL Buffer N3. Mix immediately and thoroughly by inverting the tube 4–6 times.

To avoid localised precipitation, mix the solution thoroughly, immediately after addition of Buffer N3. Large culture volumes (e.g., ≥ 5 mL) may require inverting up to 10 times. The solution should become cloudy.

If LyseBlue reagent has been used, the suspension should be mixed until all traces of blue are gone and the suspension is colourless. A homogeneous colourless suspension indicates that the SDS has been effectively precipitated.

7.2.4. Centrifuge for 10 min at 17,900 × *g* in a table-top microcentrifuge. A compact white pellet will form.

7.2.5. Apply 800 µL of the supernatant from step 7.2.4 to the QIAprep 2.0 Spin Column by pipetting.

7.2.6. Centrifuge for 60 s. Discard the flow through.

7.2.7. Wash the QIAprep 2.0 Spin Column by adding 0.5 mL Buffer PB and centrifuging for 60 s. Discard the flow through.

7.2.8. 8Wash QIAprep 2.0 Spin Column by adding 0.75 mL Buffer PE and centrifuging for 60 s.

7.2.9. Discard the flow through, and centrifuge at full speed for an additional 1 min to remove residual wash buffer.

Important: Residual wash buffer will not be completely removed unless the flow through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

7.2.10. Place the QIAprep 2.0 Spin 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 centre of each QIAprep 2.0 Spin Column, let stand for 1 min, and centrifuge for 1 min.