QIAfilter Plasmid Midi Kit (Cat #: 12243)

Things to do before starting

- Add the provided RNase A solution to Buffer P1 before use. Use one vial of RNase A (centrifuge briefly before use) per bottle of Buffer P1, to give a final concentration of 100 µg/ml.

- Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.

- **Pre-chill Buffer P3 at 4°C**.

- Optional: Add the provided LyseBlue reagent to Buffer P1 and mix before use. Use one vial LyseBlue (centrifuge briefly before use) per bottle of Buffer P1 to achieve a 1:1000 dilution. 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. For more details see “Using LyseBlue reagent” on page 15.

Quick Procedure:

1. **Pick a single colony and inoculate a starter culture of 2–5 ml LB medium containing the appropriate selective antibiotic. Incubate for approx. 8 h at 37°C with vigorous shaking (approx. 300 rpm).**

2. **Dilute the starter culture 1/500 to 1/1000 into selective LB medium. For high-copy plasmids, inoculate 25 ml medium with 25–50 µl of starter culture. For low-copy plasmids, inoculate 50–100 ml medium with 100-200 µl of starter culture. Grow at 37°C for 12–16 h with vigorous shaking (approx. 300 rpm).**

3. **Harvest the bacterial cells by centrifugation at 6000 x *g* for 15 min at 4°C.**

4. **Resuspend the bacterial pellet in 4 ml Buffer P1.**

5. **Add 4 ml Buffer P2 (Blue color appears), mix thoroughly by vigorously inverting the sealedtube 4–6 times Do not vortex (as this will result in shearing of genomic DNA), and incubate at room temperature (15–25°C) for 5 min.**

6. **Add 4 ml chilled Buffer P3 to the lysate,**

7. Pour the lysate into the barrel of the QIAfilter Cartridge. Incubate at room temperature (15–25°C) for 10 min. Do not insert the plunger!

8. Equilibrate a QIAGEN-tip 100 by applying 4 ml Buffer QBT, and allow the column to empty by gravity flow.

9. Remove the cap from the QIAfilter Cartridge outlet nozzle. Gently insert the plunger into the QIAfilter Midi and filter the cell lysate into the previously equilibrated QIAGEN-tip. Approximately 10 ml of the lysate are generally recovered

- Remove a 240µl sample of the filtered lysate and save for an analytical gel (**sample 1**) in order to determine whether growth and lysis conditions were optimal.

10. Allow the cleared lysate to enter the resin by gravity flow.

- Remove a 240µl sample from the flow-through and save for an analytical gel (**sample 2**) in order to determine the efficiency of DNA binding to the QIAGEN Resin.

11. Wash the QIAGEN-tip with 2 x 10 ml Buffer QC..

- Remove a 400 µl sample from the combined wash fractions and save for an analytical gel (**sample 3**).

12. Elute DNA with 5 ml Buffer QF.

- Remove a 100µl sample of the eluate and save for an analytical gel (**sample 4**).

13. Precipitate DNA by adding 3.5 ml (0.7 volumes) room-temperature isopropanol to the eluted DNA. Use disposable conical bottom centrifuge tubes and spin at 5000 x *g* for 60 min at 4°C.

14. Wash DNA pellet with 2 ml of room-temperature 70% ethanol, in disposable conical-bottom centrifuge tubes and spin at 5000 x *g* for 60 min at 4°C.

15. Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE buffer, pH 8.0, or 10 mM Tris·Cl, pH 8.5).

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- Optional: Add the provided LyseBlue reagent to Buffer P1 and mix before use. Use one vial LyseBlue (centrifuge briefly before use) per bottle of Buffer P1 to achieve a 1:1000 dilution. 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. For more details see “Using LyseBlue reagent” on page 15.

Procedure

1. **Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–5 ml LB medium containing the appropriate selective antibiotic. Incubate for approx. 8 h at 37°C with vigorous shaking (approx. 300 rpm).** Use a tube or flask with a volume of at least 4 times the volume of the culture.

2. **Dilute the starter culture 1/500 to 1/1000 into selective LB medium. For high-copy plasmids, inoculate 25 ml medium with 25–50 µl of starter culture. For low-copy plasmids, inoculate 50–100 ml medium with 100-200 µl of starter culture. Grow at 37°C for 12–16 h with vigorous shaking (approx. 300 rpm).** Use a flask or vessel with a volume of at least 4 times the volume of the culture. The culture should reach a cell density of approximately 3–4 x 10^9 cells per milliliter, which typically corresponds to a pellet wet weight of approximately 3 g/liter medium (see page 12).

3. **Harvest the bacterial cells by centrifugation at 6000 x *g* for 15 min at 4°C.** If you wish to stop the protocol and continue later, freeze the cell pellets at -20°C.

4. Vigorously shake the buffer bottle before use to ensure LyseBlue particles are completely resuspended and Ensure that RNase A has been added to Buffer P1. **Resuspend the bacterial pellet in 4 ml Buffer P1.** The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

5. **Add 4 ml Buffer P2 (Blue color appears), mix thoroughly by vigorously inverting the sealedtube 4–6 times Do not vortex (as this will result in shearing of genomic DNA), and incubate at room temperature (15–25°C) for 5 min.** The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 min. **During the incubation prepare the QIAfilter Cartridge: Screw the cap onto the outlet nozzle of the QIAfilter Midi or QIAfilter Maxi Cartridge. Place the QIAfilter Cartridge in a convenient tube.**

6. **Add 4 ml chilled Buffer P3 to the lysate, and mix immediately and thoroughly by vigorously inverting 4–6 times. Proceed directly to step 7. Donot incubate the lysate on ice.** A homogeneous colorless suspension indicates that the SDS has been effectively precipitated. Precipitation is enhanced by using chilled Buffer P3. The precipitated material contains genomic DNA, proteins, cell debris, and KDS.

7. **Pour the lysate into the barrel of the QIAfilter Cartridge. Incubate at room temperature (15–25°C) for 10 min. Do not insert the plunger!**

Important: This 10 min incubation at room temperature is essential for optimal performance of the QIAfilter Midi or QIAfilter Maxi Cartridge. Do not agitate the QIAfilter Cartridge during this time. A precipitate containing proteins, genomic DNA, and detergent will float and form a layer on top of the solution. This ensures convenient filtration without clogging. If, after the 10 min incubation, the precipitate has not floated to the top of the solution, carefully run a sterile pipet tip around the walls of the cartridge to dislodge it.

8. **Equilibrate a QIAGEN-tip 100 by applying 4 ml Buffer QBT, and allow the column to empty by gravity flow.** Flow of buffer will begin automatically by reduction in surface tension due to the presence of detergent in the equilibration buffer. Allow the QIAGEN-tip to drain completely. QIAGEN-tips can be left unattended, since the flow of buffer will stop when the meniscus reaches the upper frit in the column.

9. **Remove the cap from the QIAfilter Cartridge outlet nozzle. Gently insert the plunger into the QIAfilter Midi and filter the cell lysate into the previously equilibrated QIAGEN-tip.** Filter until all of the lysate has passed through the QIAfilter Cartridge, but do not apply extreme force. **Approximately 10 ml of the lysate are generally recovered** after filtration.

- Remove a 240µl sample of the filtered lysate and save for an analytical gel (**sample 1**) in order to determine whether growth and lysis conditions were optimal.

10. **Allow the cleared lysate to enter the resin by gravity flow.**

 Remove a 240µl sample from the flow-through and save for an analytical gel (**sample 2**) in order to determine the efficiency of DNA binding to the QIAGEN Resin.

11. Wash the QIAGEN-tip with 2 x 10 ml Buffer QC. Allow Buffer QC to move through the QIAGEN-tip by gravity flow. The first wash is sufficient to remove all contaminants in the majority of plasmid DNA preparations. The second wash is especially necessary when large culture volumes or bacterial strains producing large amounts of carbohydrates are used.

 Remove a 400 µl sample from the combined wash fractions and save for an analytical gel (**sample 3**).

12. Elute DNA with 5 ml Buffer QF. Collect the eluate in a 15 ml or 50 ml tube (not supplied). Use of polycarbonate centrifuge tubes is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.

Note: For constructs larger than 45–50 kb, prewarming the elution buffer to 65°C may help to increase yield.

Remove a 100µl sample of the eluate and save for an analytical gel (**sample 4**). If you wish to stop the protocol and continue later, store the eluate at 4°C. Storage periods longer than overnight are not recommended.

13. Precipitate DNA by adding 3.5 ml (0.7 volumes) room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately at ≥15,000 x *g* for 30 min at 4°C. Carefully decant the supernatant. All solutions should be at room temperature in order to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. **Alternatively, disposable conical bottom centrifuge tubes can be used for centrifugation at 5000 x *g* for 60 min at 4°C.** Isopropanol pellets have a glassy appearance and may be more difficult to see than the fluffy, salt-containing pellets that result from ethanol precipitation. Marking the outside of the tube before centrifugation allows the pellet to be more easily located. Isopropanol pellets are also more loosely attached to the side of the tube, and care should be taken when removing the supernatant.

14. Wash DNA pellet with 2 ml of room-temperature 70% ethanol, and centrifuge at ≥15,000 x *g* for 10 min. Carefully decant the supernatant without disturbing the pellet. Alternatively, disposable conical-bottom centrifuge tubes can be used for centrifugation at 5000 x *g* for 60 min at 4°C. The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

15. **Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE buffer, pH 8.0, or 10 mM Tris·Cl, pH 8.5).** Redissolve the DNA pellet by rinsing the walls to recover all the DNA, especially if glass tubes have been used. Pipetting the DNA up and down to promote resuspension may cause shearing and should be avoided. Overdrying the pellet will make the DNA difficult to redissolve. DNA dissolves best under slightly alkaline conditions; it does not easily dissolve in acidic buffers.

Determination of yield

To determine the yield, DNA concentration should be determined by both UV spectrophotometry at 260 nm and quantitative analysis on an agarose gel. **For reliable spectrophotometric DNA quantification**, ***A*260 readings should lie between 0.1 and 1.0.**

Agarose gel analysis

We recommend removing and saving aliquots during the purification procedure (samples 1–4). If the plasmid DNA is of low yield or quality, the samples can be analyzed by agarose gel electrophoresis to determine at what stage of the purification procedure the problem occurred (see page 34).