Qiagen QIAprep Spin Miniprep Kit (27104 or 27106) with a microcentrifuge

QIAgen

Abstract

This is the protocol for QIAgen's QIAprep Spin Miniprep Kit (catalog numbers 27104 or 27106) with their newer version 2.0 spin columns. For more information about the kit, see https://www.qiagen.com/us/shop/sample-technologies/dna/dna-preparation/qiaprep-spin-miniprep-kit?cmpid=QVenSPP1404quartzySC#orderinginformation.

For purification of up to 20 µg molecular biology grade plasmid DNA

- Ready-to-use plasmid DNA in minutes
- Reproducible yields of molecular biology grade plasmid DNA
- Single protocol for high- and low-copy vectors
- Even higher yields with the *High-Yield Supplementary Protocol*
- Improved QIAprep 2.0 Spin Column
- GelPilot loading dye for convenient sample analysis

The QIAprep Spin Miniprep Kit is designed for isolation of up to 20 µg high-purity plasmid or cosmid DNA for use in routine molecular biology applications, including fluorescent and radioactive sequencing and cloning. Even higher yields (up to 30 µg) can be achieved using the *High-Yield Supplementary Protocol*.

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Guidelines

- Close the bottle containing Buffer P2 immediately after use to avoid acidification of Buffer P2 from CO2 in the air.
- All centrifugation steps are carried out at 13,000 rpm (~17,900 x g) in a conventional, table-top microcentrifuge.
- Ensure that the elution buffer is dispensed directly onto the center of the QIAprep membrane for optimal elution of DNA. Average eluate volume is 48 µl from an elution buffer volume of 50 µl (QIAprep spin procedures), and 60 µl from an elution buffer volume of 100 µl (QIAprep

multiwell procedures).
- For increased DNA yield, use a higher elution-buffer volume.
- For increased DNA concentration, use a lower elution-buffer volume
- If water is used for elution, make sure that its pH is between 7.0 and 8.5. Elution efficiency is dependent on pH and the maximum elution efficiency is achieved within this range. A pH <7.0 can decrease yield. Note: Store DNA at –20°C when eluted with water, as DNA may degrade in the absence of a buffering agent. DNA can also be eluted in TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

Before start

Growth of bacterial cultures in tubes or flasks

Pick a single colony from a freshly streaked selective plate and inoculate a culture of 1–5 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 since cells begin to lyse and plasmid yields may be reduced. Use a tube or flask with a volume of at least 4 times the volume of the culture.

- 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.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- Check Buffers P2 and N3 before use for salt precipitation. Redissolve any precipitate by warming to 37°C. Do not shake Buffer P2 vigorously.
- Optional: 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.

Protocol

Lysis

Step 1.

Resuspend pelleted bacterial cells in 250µl Buffer P1 and transfer to a micro-centrifuge tube.

AMOUNT

250 µl Additional info:

NOTES

Harold Bien 15 Nov 2015

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, 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.

ANNOTATIONS
Petra Visic 29 Jun 2016

If pelleted bacterial cells are not resuspending easily (too much pellet), increase amount of Buffer P1.

Lysis

Step 2.
Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times.

AMOUNT
250 µl Additional info:

NOTES
Harold Bien 15 Nov 2015

Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, 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 localized colorless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

Lysis

Step 3.
Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times.

AMOUNT
350 µl Additional info:

NOTES
Harold Bien 15 Nov 2015

To avoid localized 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 trace of blue has gone and the suspension is colorless. A homogeneous colorless sus- pension indicates that the SDS has been effectively precipitated.

Lysis

Step 4.
Centrifuge for 10 min at 13,000 rpm (17,900 x *g*) in a table-top microcentrifuge.

DURATION
00:10:00

Binding

Step 5.
Apply the supernatants from step 4 to the QIAprep spin column by decanting or pipetting.

NOTES
Alan Cone 10 Jul 2015

Roughly about 850 uL.

Binding

Step 6.

Centrifuge for 30–60 s. Discard the flow-through.

DURATION

00:01:00

Wash

Step 7.

Wash the QIAprep spin column by adding 0.5 ml Buffer PB and centrifuging for 30–60 s. Discard the flow-through.

AMOUNT

500 µl Additional info:

DURATION

00:01:00

NOTES

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This step is necessary to remove trace nuclease activity when using endA+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5®α do not require this additional wash step.

Wash

Step 8.

Wash QIAprep spin column by adding 0.75 ml Buffer PE and centrifuging for 30–60 s.

AMOUNT

750 µl Additional info:

DURATION

00:01:00

Wash

Step 9.

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

DURATION

00:01:00

NOTES

Harold Bien 15 Nov 2015

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.

Elution

Step 10.

Place the QIAprep column in a clean 1.5 ml microcentrifuge tube.

DURATION

00:01:00

Elution

Step 11.

Centrifuge for 1 min.

DURATION
00:01:00

Elution

Step 12.
Let stand 1 minute

DURATION
00:01:00

Lysis

Step 13.
Centrifuge 1-5mL bacterial overnight culture at >6800g for 3 minutes at room temperature

AMOUNT
5 ml Additional info:

Warnings

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. Buffers P2, N3, and PB contain irritants. Wear gloves when handling these buffers. For more information, please consult the appropriate safety data sheets (SDSs).

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Buffers N3 and PB contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.