



QuickPick™ Plasmid DNA

52001D • plasmid DNA purification kit, 8 preps

52011 • plasmid DNA purification kit, 48 preps

52021 • plasmid DNA purification kit, 96 preps

INTRODUCTION

These are the instructions for use for the QuickPick™ Plasmid DNA purification kit. Please read the entire instructions carefully before starting to work with the reagents. The QuickPick reagents are intended for use with the PickPen® magnetic tools supplied by Bio-Nobile. Also refer to the PickPen® instructions for use. PickPen® 1-M is recommended when working in microtube format and PickPen® 8-M when working in microplate format typically with smaller sample sizes and higher throughput.

The QuickPick Plasmid DNA purification kit provides a fast and simple means of isolating plasmid DNA from bacteria cells. The technique does not require any organic solvents and eliminates the need for repeated centrifugation, vacuum filtration or column separation. The plasmid DNA purified by the QuickPick Plasmid DNA kit is ready for downstream applications.

SPECIFICATIONS

Vessel format:	1.5 ml microtubes, 96-well microplates (U-bottom, minimum volume of 300 µl is recommended)
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Starting material per preparation:	1.5 ml <i>E. coli</i> cells (OD ₆₀₀ = 2-4, approx. 2-4 x10 ⁹ cells)
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Elution volume:	40 µl
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Typical yield per preparation: (yields depend on the plasmid used, growth conditions etc.)	1.5 µg - 11 µg
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Purity:	≥ 1.8*
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Total protocol time:	with PickPen® 1-M, 1 prep: < 20 min (including sample preparation)
	with PickPen® 8-M, 8 preps: < 35 min (including sample preparation)

* Ratio of absorbance at 260/280 nm is corrected with absorbance at 320 nm

KIT CONTENTS	52001D	52011	52021
Plasmid DNA MagaZorb™ (Magnetic Particles):* **	40 µl	250 µl	500 µl
Plasmid DNA Buffer A, Store at +2 - +8 °C:	340 µl	2.0 ml	4.0 ml
Plasmid DNA Buffer B:	340 µl	2.0 ml	4.0 ml
Plasmid DNA Buffer C:	340 µl	2.0 ml	4.0 ml
Plasmid DNA Binding Buffer: **	1.0 ml	6.3 ml	12.6 ml
Plasmid DNA Wash Buffer: **	1.9 ml	11 ml	22.0 ml
Plasmid DNA Elution Buffer:	340 µl	2.0 ml	4.0 ml
8-Pack PickPen® tips:	1 pack	6 packs	12 packs

*MagaZorb™ technology is a trademark of Cortex Biochem Inc.

** Reagents contain 0.02% NaN₃

ADDITIONAL MATERIAL REQUIRED BUT NOT SUPPLIED WITH THE KIT

1. PickPen® 1-M or PickPen® 8-M magnetic tool. See also the PickPen® instructions for use.
2. Microcentrifuge
3. Sterile microtubes or 96-well microplates (U-bottom)
4. Sterile aerosol resistant micropipettor tips (recommended)
5. Tube rotator (for microtubes), or orbital shaker (for microplates).

PRINCIPLE

The QuickPick Plasmid DNA purification protocol is based on a modified alkaline lysis procedure followed by binding of the plasmid DNA to Plasmid DNA MagaZorb™ Magnetic Particles in the presence of Plasmid DNA Binding Buffer. Plasmid DNA MagaZorb™ Magnetic Particles with the bound plasmid DNA are captured with PickPen® and contaminants are removed by washing with Plasmid DNA Wash Buffer. The plasmid DNA is then eluted from the magnetic particles with the Plasmid DNA Elution Buffer. The procedure starting from sample preparation and ending with purified plasmid DNA lasts less than 20 minutes.

PROCEDURE

PickPen® tips

The PickPen® tips in the 8-Pack are RNase/DNase free and are ready to use.

Note: Tips should be picked up gently from the pack. Too much pressure may open the pack.

The tips packed in bulk quantities in plastic bags are not RNase/DNase free. The tips can be autoclaved (+121 °C at least 20 min) or baked (+180 °C overnight) provided that they are first removed from the plastic bag or the pack. The separately available PickPen® tip box can also be autoclaved.

Sample preparation from *E. coli* cells

Example: preparation of 1-8 samples

1. Harvest 1-8 x 1.5 ml of bacterial cell cultures by centrifuging for 2 minutes at 18,000 x g and discard the supernatants. A short spin of pelleted cells may be performed to remove any residual medium to avoid decrease in yields. Resuspend pelleted bacterial cells in 40 µl of Buffer A by vortexing, pipetting up and down or scraping the tubes across the holes of a tube rack (see Ref. 1). No cell clumps should be visible after resuspension of the pellets. Transfer the suspensions into clean tubes.
2. Add 40 µl of Buffer B (lysis) into consecutive sample tubes every 30 seconds and mix by inverting the tubes 4-6 times. Do not vortex. If necessary, continue inverting the tubes until the solutions become viscous and slightly clear. Allow the lysis reactions to proceed for at least 2 minutes, but do not let them exceed 5 minutes.
3. Add 40 µl of Buffer C into the tubes in the same order as in step 2. Invert the tubes immediately but gently 4-6 times. The solutions should become cloudy with a visible white precipitate.
4. Centrifuge for 2 – 10 minutes at 18,000 x g. A white pellet should form. If the supernatants contain large amounts of floating particulates after centrifugation repeat the centrifugation step before proceeding and use only the clear supernatants as sample. Proceed immediately with the protocol.

REFERENCE 1

1. Voo, K.S. and Jacobsen, B.M. (1998) Rapid resuspension of pelleted bacterial cells for miniprep plasmid DNA isolation. *Biotechniques*. 2, 240-3.

NOTES TO THE PROTOCOL:

1. Due to the small reagent volumes in kit 52001D, all reagent tubes should be spun briefly before pipetting to get all the drops from the caps into the tubes.
2. When using microplates, the use of an orbital shaker is recommended for mixing during incubations. Adjust the speed to the highest possible level without causing liquid overflow but still keeps magnetic particles in suspension.
3. The yields may vary depending on the target plasmid characteristics. Also the growth medium type and growth conditions may affect the amount of plasmid DNA obtained.
4. If concentrated plasmid solutions are needed the volume of the elution buffer may be reduced. Volumes less than 10 µl are not recommended.
5. **Note for QuickPick™ Plasmid DNA kit protocol with PickPen® 8-M:** Higher plasmid DNA yield can be obtained by using 125 µl of Binding Buffer. In this case microplates with well volume >300 µl should be used.

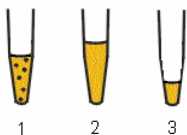
QuickPick™ Plasmid DNA kit protocol with PickPen® 1-M

All solutions should be clear when used. If precipitates have formed warm the solutions gently until the precipitates have dissolved. Plasmid DNA MagaZorb™ Magnetic Particles should be mixed thoroughly just before pipetting. Vortexing of the magnetic particles is not recommended. Repeat pipettors should not be used when dispensing magnetic particles.

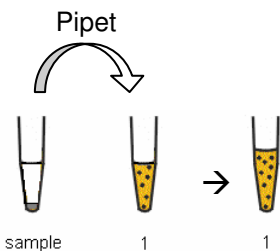
Protocol:

1. Number tubes from 1 to 3 and pipette QuickPick Plasmid DNA kit reagents into tubes as follows:

Tube 1:	5 μ l	Plasmid DNA MagaZorb™ Magnetic Particles
	125 μ l	Plasmid DNA Binding Buffer
Tube 2:	200 μ l	Plasmid DNA Wash Buffer
Tube 3:	40 μ l	Plasmid DNA Elution Buffer



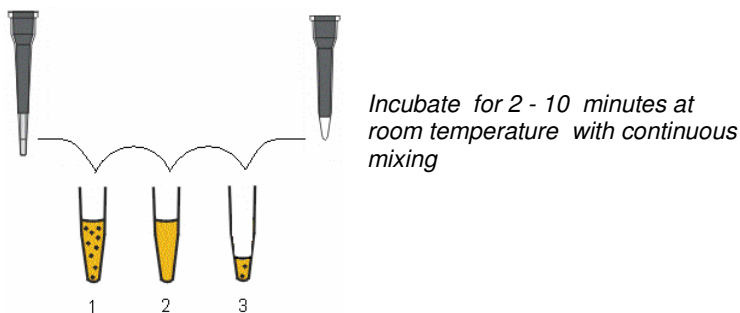
2. Carefully transfer the clear sample supernatant (from step 4 in: **“Sample preparation from *E. coli* cells”**) into a tube 1, which already contains the Binding Buffer and the Magnetic Particles. Incubate for 5 - 10 minutes at room temperature, while mixing continuously (using a tube rotator, vortex or manually). Make sure that the particles are in suspension during this step. Mixing is essential for maximizing the plasmid DNA binding to the magnetic particles.



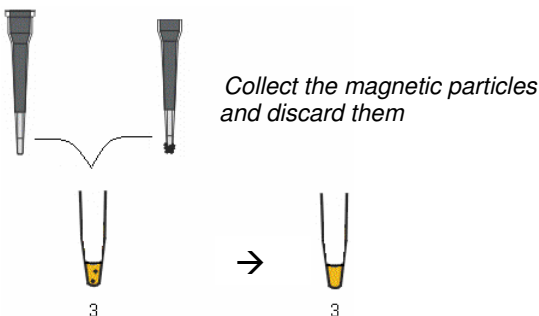
Incubate for 5 - 10 minutes at room temperature with continuous mixing

3. Pick up the PickPen® tip using the PickPen® 1-M. Extend the magnet 2-3 times to check that the tip is firmly in place. Collect the magnetic particles from tube 1 with PickPen® and release them into tube 2 (Wash buffer). Mix the suspension for 5 - 10 seconds using the PickPen® tip. Note that the magnet has to be withdrawn at this point.

4. Collect the magnetic particles from tube 2 and release them into tube 3 (Elution buffer). Incubate for 2 - 10 minutes at room temperature while mixing continuously (using a tube rotator, vortex or manually).



5. Collect the magnetic particles from tube 3 and discard them and the tip. The eluate in tube 3 contains the isolated plasmid DNA and is ready to be used in downstream applications. If the purified plasmid DNA is not used on the same day, store at -20°C until use.



QuickPick™ Plasmid DNA kit protocol with PickPen® 8-M

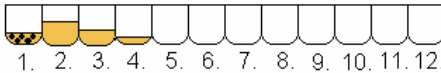
All solutions should be clear when used. If precipitates have formed warm the solutions gently until the precipitates have dissolved. Plasmid DNA MagaZorb™ Magnetic Particles should be mixed thoroughly just before pipetting. Vortexing of the magnetic particles is not recommended. Repeat or 8-channel pipettors should not be used when dispensing magnetic particles.

The following instructions are for 8 parallel samples. Samples are lysed in microtubes and transferred into microplates (U-bottom) where the rest of the protocol is carried out.

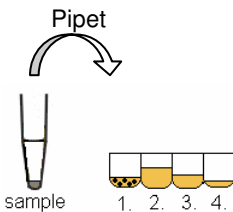
Protocol:

1. Pipette QuickPick™ Plasmid DNA kit reagents into rows 1-4 as follows:

Row 1:	5 μ l	Plasmid DNA MagaZorb™ Magnetic Particles
	100 μ l	Plasmid DNA Binding Buffer
Row 2:	150 μ l	Plasmid DNA Wash Buffer
Row 3:	75 μ l	Plasmid DNA Wash Buffer
Row 4:	40 μ l	Plasmid DNA Elution Buffer

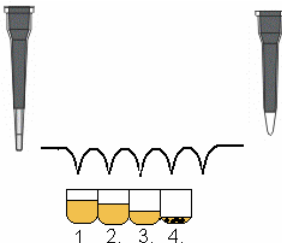


2. Carefully transfer the clear sample supernatants (from step 4 in: “**Sample preparation from *E. coli* cells**”) into row 1, which already contains the Binding Buffer and the Magnetic Particles. Mix and incubate for 5 – 10 minutes at room temperature, while mixing continuously on the orbital shaker. Make sure that the particles are in a suspension during this step. Continuous mixing during this binding step is essential for maximizing the plasmid DNA binding to the magnetic particles.



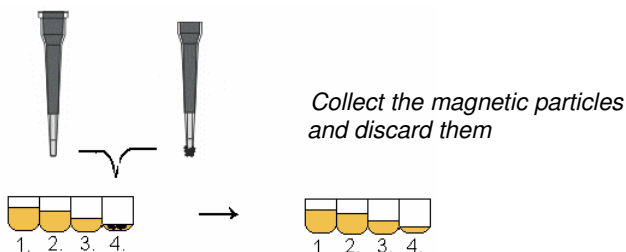
Incubate for 5 - 10 minutes at room temperature with continuous mixing

3. Pick up the PickPen® tips using PickPen® 8-M. Collect the magnetic particles from row 1 with PickPen® and release them into row 2 (Wash Buffer). Mix the suspension for 5 - 10 seconds using PickPen® tips. Note that the magnets have to be withdrawn at this point. Repeat the washing step in row 3.
4. Collect the magnetic particles from row 3 and release them into row 4 (Elution Buffer). Incubate for 2 - 10 minutes at room temperature while mixing the microplate on the orbital shaker at room temperature.



Incubate for 2 - 10 minutes at room temperature with continuous mixing

5. Collect the magnetic particles from row 4 and discard them and the tips. The eluates in row 4 now contain the isolated plasmid DNA and are ready to be used in downstream applications. If the purified plasmid DNA is not used on the same day, store at -20°C until use.



Processing multiple samples with PickPen® 8-M simultaneously, example:

The following instructions are for 24 parallel samples processed on 1 microplate. Samples 1-8 are processed in rows 1-4, samples 9-16 are processed in rows 5-8 and samples 17-24 in rows 9-12.

When processing 24 samples simultaneously, pay attention to following notes during the sample preparation (See also “**Sample preparation from *E. coli* cells**”):

1. All 24 samples can be suspended in Buffer A at the same time and stored for up to 2 hours at room temperature.
2. Since the lysis reaction in Buffer B may not exceed 5 minutes, proceed with steps 2 and 3 with at most 8 samples at a time.
3. Centrifuge all 24 samples at the same time, as described in step 4.

Protocol:

1. Prepare 24 samples as described previously. During the sample centrifugation step pipette the QuickPick Plasmid DNA reagents to the microplate as described in the protocol; reagents for samples 1-8 in rows 1-4, for samples 9-16 in rows 5-8 and for samples 17-24 in rows 9-12. Number PickPen® 8-Packs from 1 to 3; pack 1 is for samples 1-8, pack 2 for samples 9-16 and pack 3 for samples 17-24.
2. Carefully transfer the clear sample supernatants from tubes 1-8 into the respective wells of row 1 (Binding Buffer, Magnetic Particles). Proceed similarly with samples 9-24 in rows 5 and 9. Mix the microplate on the orbital shaker for 5 – 10 minutes at room temperature. Make sure that the particles stay in suspension during this step.
3. Pick up the PickPen® tips from 8-Pack nr 1 using PickPen® 8-M. Collect the magnetic particles from row 1 (samples 1-8) and release them into row 2 (Wash Buffer). Mix the suspensions briefly using the PickPen® tips. Repeat the washing step in row 3 (Wash Buffer). Collect the magnetic particles from row 3 and release them into row 4 (Elution Buffer).

4. Release the PickPen[®] tips into 8-Pack nr 1 and store while handling the next rows of samples. Pick up PickPen[®] tips from 8-Pack nr 2. Collect the magnetic particles from row 5 (samples 9-16) and carry out the washing steps (rows 6 and 7) as described above. Finally release the magnetic particles into row 8 (Elution Buffer). Proceed similarly with samples 17-24 with PickPen[®] tips from 8-Pack nr 3 in rows 9-12.
5. When the magnetic particles are in rows 4, 8 and 12 respectively, mix the microplate on the orbital shaker at room temperature for 2 - 10 minutes.
6. Pick up the PickPen[®] tips from 8-Pack nr 1. Collect the magnetic particles from row 4 and discard them and the tips. Proceed similarly with samples 9-24 in rows 8 and 12 respectively, using the tips from packs 2 and 3. The eluates containing the plasmid DNA are ready for downstream applications or storage.

STORAGE AND STABILITY

The QuickPick™ Plasmid DNA purification kit should be stored at room temperature, except Plasmid DNA Buffer A, which should be stored at +2 - +8°C. Magnetic particles should not be frozen.

WARNINGS AND LIMITATIONS

The QuickPick™ Plasmid DNA purification kit is intended for research use only, and is not intended for use in human diagnostic or therapeutic procedures. Standard methods for preventing contamination with DNases during preparation of DNA must be taken. Precautions should also be taken to avoid contamination of opened vials. Do not pipette by mouth.

Plasmid DNA MagaZorb™ reagent, Wash Buffer and Binding Buffer contain 0.02 % sodium azide (NaN₃) as a preservative. When in contact with acid or heavy metal ions, it forms a highly toxic gas. Preservatives such as NaN₃ are toxic if ingested. Do not pipette by mouth. Direct skin contact must be avoided. Appropriate precautions should be taken when handling these solutions.

DISCLAIMERS AND WARRANTIES

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Innovations for magnetic bioseparations

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