PRODUCTS&SERVICES
Innovations for magnetic bioseparations

INSTRUCTIONS FOR USE

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 QuicPick™ magnetic tools supplied by BN Products & Services. Also refer to the tool instructions for use. QuicPick one magnet is recommended when working in microtube format and QuicPick multiEight 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)
Sample material per preparation:	1.5 ml *E. coli* cells (OD_{600} = 2-4, approx. 2-4 x10^9 cells)
Elution volume:	40 µl
Typical yield per preparation:	1.5 µg - 11 µg
Yields depend on the plasmid used, growth conditions etc.	
Purity:	≥ 1.8*
Total protocol time: with QuicPick, 1 prep:	< 20 min (including sample preparation)
with QuicPick multiEight, 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 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

* Reagents contain 0.02% NaN_3

ADDITIONAL MATERIAL REQUIRED BUT NOT SUPPLIED WITH THE KIT

1. QuicPick one magnet or QuicPick multiEight magnetic tool
2. QuicPick tips
3. Microcentrifuge
4. Sterile microtubes or 96-well microplates (U-bottom)
5. Sterile aerosol resistant micropipettor tips (recommended)
6. 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 magnetic particles in the presence of Plasmid DNA Binding buffer. Plasmid DNA magnetic particles with the bound plasmid DNA are captured with QuicPick® 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

QuicPick tips

The tips packed in bulk quantities in plastic bags are clean but not guaranteed to be RNase/DNase free. RNAse free tips can be purchased boxed. The tips can be autoclaved (+121 °C at least 20 min) The separately available 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 spinned 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. **Note**: for QuickPick™ Plasmid DNA kit protocol with QuicPick multiEight®: 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 QuicPick one magnet

All solutions should be clear when used. If precipitates have formed warm the solutions gently until the precipitates have dissolved. Plasmid DNA 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.

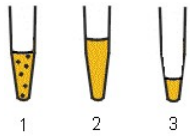
Protocol:

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

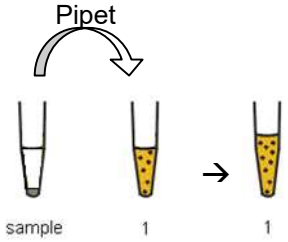
Tube 1: 5 µl Plasmid DNA magnetic particles plus 125 µl Plasmid DNA Binding buffer

Tube 2: 200 µl Plasmid DNA Wash buffer

Tube 3: 40 µl Plasmid DNA Elution buffer



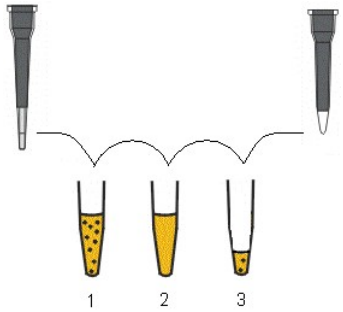
2. Carefully transfer the clear sample supernatant (from step 4 in: "**Sample preparation from *E. coli* cells**") into a tube 1, which already contains the Binding buffer and the Magnetic Particles. Incubate for 5 - 10 minutes at room temperature, while mixing continuously (using a tube rotator, vortex 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

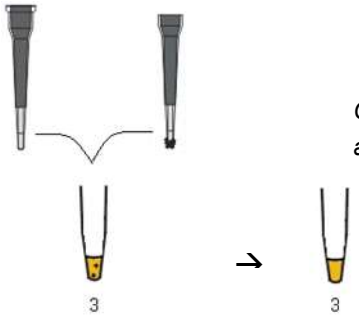
3. Pick up the tip using the tool. Extend the magnet 2-3 times to check that the tip is firmly in place. Collect the magnetic particles from tube 1 with tool and release them into tube 2 (Wash buffer). Mix the suspension for 5 - 10 seconds using the tip. Note that the magnet has to be withdrawn at this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



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

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.



Collect the magnetic particles and discard them

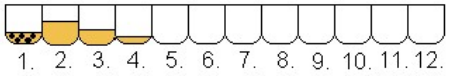
QuickPick™ Plasmid DNA kit protocol with QuicPick multiEight

All solutions should be clear when used. If precipitates have formed warm the solutions gently until the precipitates have dissolved. Plasmid DNA 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.

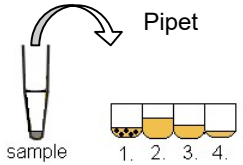
Protocol:

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

Row 1:	5 µl	Plasmid DNA magnetic particles
	100 µl	Plasmid DNA Binding buffer
Row 2:	150 µl	Plasmid DNA Wash buffer
Row 3:	75 µl	Plasmid DNA Wash buffer
Row 4:	40 µl	Plasmid DNA Elution buffer



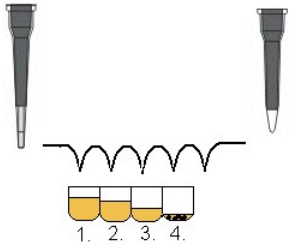
2. Carefully transfer the clear sample supernatants (from step 4 in: "**Sample preparation from *E. coli* cells**") into row 1, which already contains the Binding buffer and the Magnetic Particles. Mix and incubate for 5 – 10 minutes at room temperature, while mixing continuously on the orbital shaker. 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

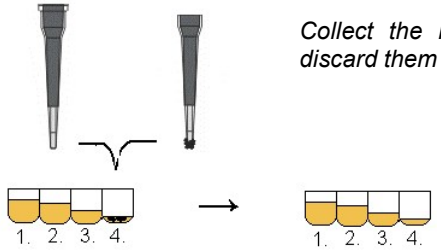
3. Pick up the QuicPick® tips using QuicPick multiEight®. Collect the magnetic particles from row 1 with QuicPick multiEight® and release them into row 2 (Wash buffer). Mix the suspension for 5 - 10 seconds usingQuicPick® tips. Note that the magnets have to be withdrawn at this point. Repeat the washing 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.



Collect the magnetic particles and discard them

Processing multiple samples with QuicPick multiEight 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**"):

All 24 samples can be suspended in buffer A at the same time and stored for up to 2 hours at room temperature. 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.

Centrifuge all 24 samples at the same time, as described in step 4.

Protocol:

1. Load 24 tips into a suitable tip box.
2. 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.
3. 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.
4. Pick up 8 QuicPick® tips from a tip box with the QuicPick multiEight® . Collect the magnetic particles from row 1 (samples 1-8) and release them into row 2 (Wash buffer). Mix the suspensions briefly using the QuicPick® 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).
5. Release the QuicPick® tips back into the tip box and store while handling the next rows of samples. Pick up eight new QuicPick® tips from the tip box. 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 new QuicPick® tips in rows 9-12.
6. 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.
7. Pick up the first set of QuicPick® tips from the box. 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 the next rows in the tip box. 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 magnetic particles, Wash buffer and Binding buffer contain 0.02 % sodium azide (NaN_3) as a preservative. When in contact with acid or heavy metal ions, it forms a highly toxic gas. Preservatives such as NaN_3 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

BN Products & Services warrants that its products shall be free from defects in materials and workmanship and shall meet performance specifications if stored and used in accordance with the instructions for use, for a period up to the expiry date provided on the kit package. This warranty does not cover normal wear and tear or misuse of the product. BN Products & Services' obligation and the purchaser's exclusive remedy under this warranty is limited to replacement, at BN Products & Services' expense, of any products defective in manufacture. In no event shall BN Products & Services be liable for any special, incidental or consequential damages. This warranty statement may be subject to modification in accordance with local laws, regulations and business practices.

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