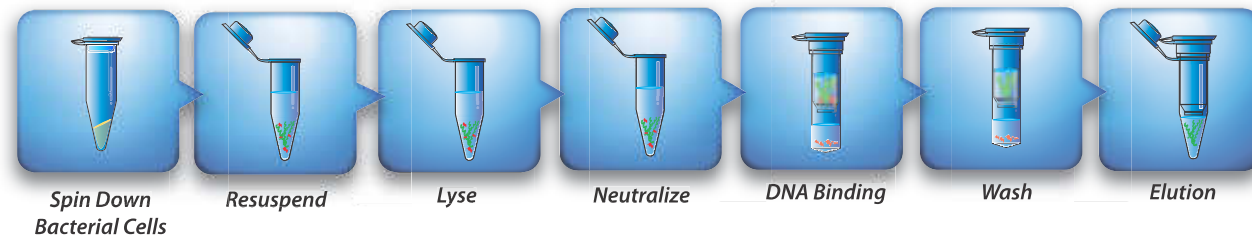
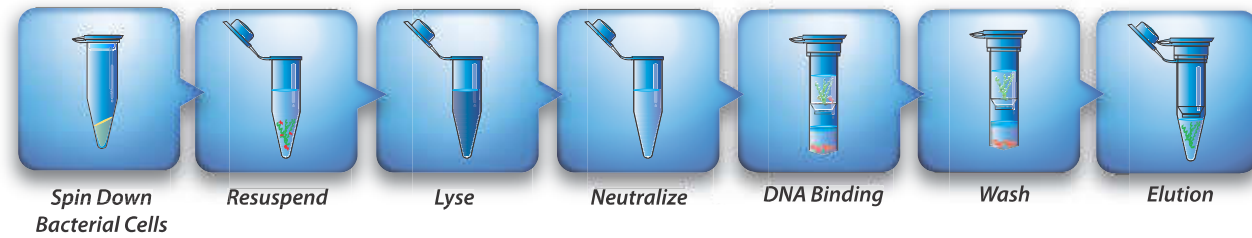




Workflow without using BlueMix Lysis Buffer



Workflow while using BlueMix Lysis Buffer



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HiYield Plus™ Plasmid Mini Kit Protocol Book

High Yield Plasmid DNA from 1-7 ml of Bacterial Cultures

Cat. No. QPD100 / QPD300

Index

HiYield Plus™ Plasmid Mini Kit

— Description	2
— Storage	2
— Important Notes	3
— Protocol	5
— Troubleshooting	9



Cat. No. QPD100

100 mini preps / kit

PD1 Buffer: 25 ml *

PD2 Buffer: 25 ml **

PD3 Buffer: 45 ml

BlueMix Lysis Buffer: 250 µl

W1 Buffer: 45 ml

Wash Buffer (concentrated): 25 ml ***

Elution Buffer: 6 ml

RNase A: 100 µl

QPD Column: 100 pcs

2 ml Collection Tube: 100 pcs

Cat. No. QPD300

300 mini preps / kit

PD1 Buffer: 65 ml*

PD2 Buffer: 75 ml **

PD3 Buffer: 100 ml

BlueMix Lysis Buffer: 650 µl

W1 Buffer: 130 ml

Wash Buffer (concentrated): 50 ml ***

Elution Buffer: 30 ml

RNase A: 260 µl

QPD Column: 300 pcs

2 ml Collection Tube: 300 pcs

Sample: 1-7 ml of Bacterial Cultures

Yield: Up to 50 µg of pure plasmid DNA

Format: Spin Column

Operation Time: Within 15 Minutes

Elution Volume: 30-100 µl

Mix following buffers prior to the initial use:

* Add provided RNase A to PD1 Buffer and mix by shaking for a few seconds. Check the box on the bottle label showing RNase A is added. After addition of RNase A and optional BlueMix Lysis Buffer, PD1 Buffer is stable for 6 months when stored at 2-8°C.

** If precipitates have formed in PD2 Buffer, warm the buffer in a 37°C waterbath, followed by gentle shaking to dissolve before use.

*** Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to the initial use.

1

Description

HiYield Plus™ Plasmid Mini Kit is designed for rapid isolation of up to 50 µg of plasmid DNA from 1-7 ml of bacterial cultures without phenol extraction and alcohol precipitation. The entire procedure can be completed within 15 minutes. BlueMix Lysis Buffer (an optional color indicator) is included in this kit to provide visual identification of optimum buffer mixing and prevent common handling errors. BlueMix Lysis Buffer is ideal for use by scientists who want to be assured of maximum product yield. Typical yields are 20-35 µg for high-copy plasmid or 3-10 µg for low-copy plasmid from 4 ml of bacterial cultures. The purified plasmid DNA is ready for use in many downstream applications. For large scale plasmid purification, please refer to HiYield™ Plasmid Midi Kit, ion exchange based Fastlon™ Plasmid Midi/Maxi Kits or Fastlon Plus™ Plasmid Midi Kits.

Features

Purification of up to 50 µg of pure plasmid DNA within 15 minutes.

BlueMix Lysis Buffer provides visual identification of optimum buffer mixing and prevent common handling errors.

Phenol, chloroform or alcohol are not required.

Applications

Purified DNA is ready for direct use in restriction enzyme digestion, ligation, transformation, PCR, DNA sequencing, DNA library screening and analysis.

Quality Control

The quality of HiYield Plus™ Plasmid Mini Kits are tested on a lot-to-lot basis by isolation of plasmid DNA from 4 ml culture of E.coli DH5a transformed with the plasmid pBluescript ($A_{600} > 2$ units/ml). Purified DNA is quantified with a spectrophotometer and the yield of plasmid DNA is more than 20 µg with A_{260}/A_{280} ratio 1.8 to 2.0. The purified DNA is used in Eco R1 digestion and checked by electrophoresis.

Storage

HiYield Plus™ Plasmid Mini Kits should be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. After addition of RNase A and optional BlueMix Lysis Buffer, PD1 Buffer is stable for 6 months when stored at 2-8°C. RNase A stock solution can be stored for 1 year at room temperature.

2

Important Notes

Please read the entire notes before starting any of the protocol procedures.

Growth of Bacterial Cultures

1. Using bacterial culture (O.D.600 of 2-6) is recommended.
2. Use fresh bacterial cultures only. Incubate for 12-16 hours at 37°C with 150-180 rpm shaking.
3. Do not use overgrown bacterial cultures. Growth for more than 16 hours is not recommended.
4. Solid medium and liquid medium (i.e. LB medium) should contain an antibiotic, such as ampicillin.

Buffer Notes

1. Before use, briefly centrifuge the provided RNase A and add entire RNase A to PD1 Buffer. Shake the mixture for a few seconds and store the mixture at 2-8°C. Check the box on the bottle label showing RNase A is added. After addition of RNase A and optional BlueMix Lysis Buffer, PD1 Buffer is stable for 6 months when stored at 2-8°C.
2. Check PD2 Buffer before use. Dissolve any precipitate by warming to 37°C, followed by gentle shaking.
3. Add absolute ethanol (see the bottle label for volume) to Wash Buffer and mix by shaking for a few seconds. Check the box on the bottle label showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.
4. Most buffers contain irritants. Always wear a lab coat, disposable gloves, and protective goggles when handling these buffers.
5. Optional: Add the provided BlueMix Lysis Buffer to PD1 Buffer (RNase A added) and mix before use. BlueMix Lysis Buffer should be added to PD1 Buffer at a ratio of 1:100 to achieve the optimal working concentration. e.g., 2 µl of BlueMix Lysis Buffer into 200 µl of PD1 Buffer. Using a simple color indicator, BlueMix Lysis Buffer prevents common handling errors, ensuring efficient cell lysis and neutralization.

Centrifugation Notes

1. All centrifugation steps are carried out at 14,000-1,6000 xg in a conventional, table-top microcentrifuge.
2. If using >5 ml of bacterial cells, centrifuge at 16,000-20,000 xg for 5-8 minutes in neutralization step.

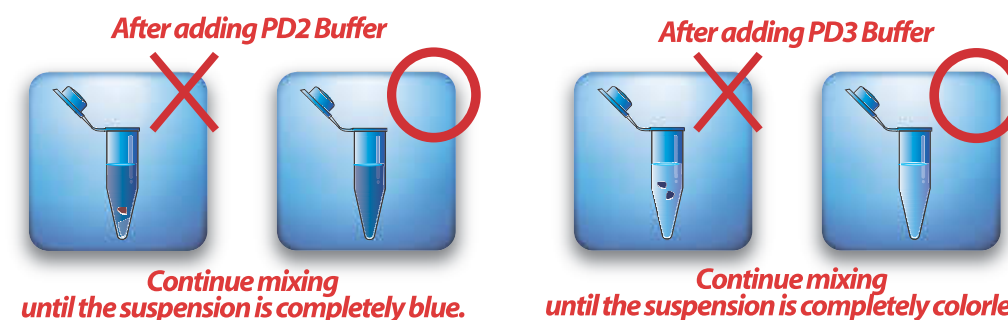
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Elution Notes

1. Ensure that the elution buffer, TE or water is dispensed directly onto the center of the QPD Column in a 2 ml Collection Tube.
2. To increase DNA yield, use a higher elution buffer volume. To increase DNA concentration, use a lower elution buffer volume.
3. If plasmid DNA are larger than 10 kb, use pre-heated Elution Buffer (60-70°C).
4. Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. If plasmid DNA are larger than 10 kb, use pre-heated TE (60-70°C).
5. If using water for elution, ensure its pH is ≥ 8.0 . ddH₂O should be fresh as ambient CO₂ can quickly cause acidification. Ensure that water is added into the center of the QPD Column matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated water (60-70°C).

How BlueMix Lysis Buffer Works

1. BlueMix Lysis Buffer precipitates after addition into PD1 Buffer.
2. Precipitates will be completely dissolved after addition of PD2 Buffer. The color of the suspension changes to blue. If colorless regions or brownish cell clumps are still visible, continue mixing until the suspension is completely blue.
3. After adding PD3 Buffer, the suspension turns colorless. If blue regions remain in the suspension, continue mixing until it becomes completely colorless. No traces of blue indicates correct mixing.

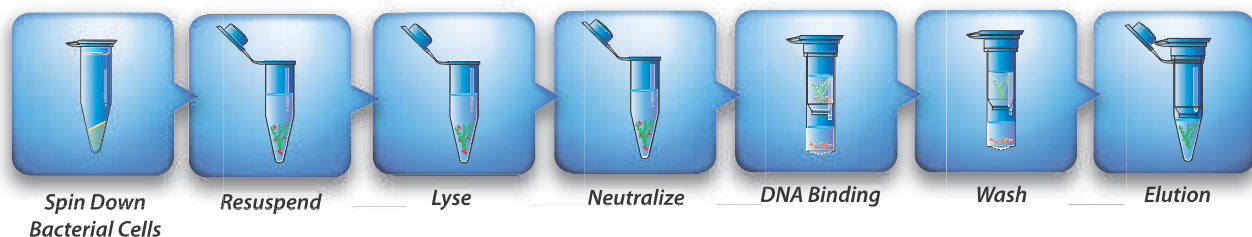
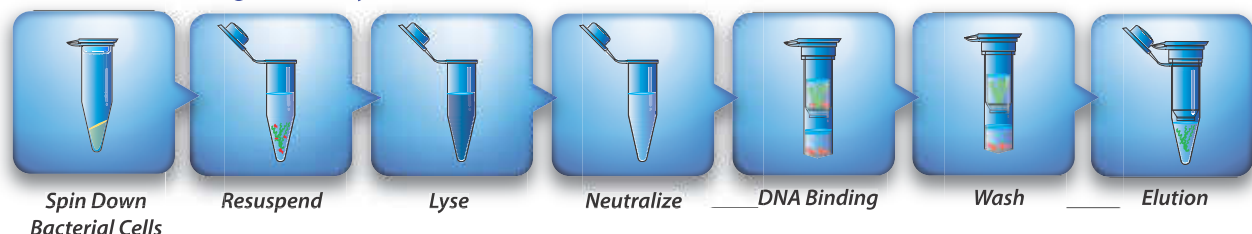


4

Protocol

Please read the entire important notes before starting any of the protocol procedures.

This protocol is designed for preparation of up to 35 µg of high-copy plasmid or 10 µg of low-copy plasmid using HiYield Plus™ Plasmid Mini Kit. The typical yield is about 20-35 µg of high-copy-number plasmid or 3-10 µg of low-copy plasmid when preparing 4 ml of cultured bacterial cells from overnight bacterial culture in LB medium. If the plasmid is larger than 10 kb, preheat the Elution Buffer to 70°C prior to the Elution Step.

Workflow without using BlueMix Lysis Buffer**Workflow while using BlueMix Lysis Buffer**

Use of BlueMix Lysis Buffer is optional and is not required to successfully perform plasmid DNA extraction. BlueMix Lysis Buffer is a color indicator included in this kit to provide visual identification of optimum buffer mixing and prevent common handling errors.

Things to do before starting

- 1) Briefly centrifuge the provided RNase A and add entire RNase A to PD1 Buffer. Shake the mixture for a few seconds and store the mixture at 2–8°C. Check the box on the bottle label showing the RNase A is added. After addition of RNase A and optional BlueMix Lysis Buffer, PD1 Buffer is stable for 6 months when stored at 2–8°C.
- 2) Check PD2 Buffer before use. Dissolve any precipitate by warming to 37°C, followed by gentle shaking.
- 3) Add absolute ethanol (see the bottle label for volume) to Wash Buffer and mix by shaking for a few seconds. Check the box on the bottle label showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.

Harvesting the bacterial cells

1. Transfer 1.5 ml of cultured bacterial cells (1-2 x 10⁹ E. coli grown in LB medium) to a 1.5 ml microcentrifuge tube. Centrifuge at 14,000-16,000 x g for 1 minute at room temperature to form a cell pellet. Then discard the supernatant completely. Use a narrow pipette tip to ensure the supernatant is completely removed. Repeat the harvesting step as required. If more than 1.5 ml of cells are used, repeat the harvesting step. For samples volume between 1.5-7.0 ml, use the same 1.5 ml microcentrifuge tube.

Resuspension**Steps without using BlueMix Lysis Buffer**

2. Add 200 µl of PD1 Buffer (RNase A added) to the 1.5 ml microcentrifuge tube containing the cell pellet.
3. Resuspend the cell pellet completely by vortex or pipette. Continue to vortex or pipette until all traces of the cell pellet have been dissolved.

Steps while using BlueMix Lysis Buffer

2. Add 200 µl of PD1 Buffer (RNase A added) to a new 1.5 ml microcentrifuge tube. Add 2 µl of BlueMix Lysis Buffer to the same 1.5 ml microcentrifuge tube then mix by shaking gently.

- It is normal for precipitates to form after mixing BlueMix Lysis Buffer with PD1 Buffer. BlueMix Lysis Buffer should be added to PD1 Buffer at a ratio of 1:100 to achieve the optimal working concentration. e.g., 2 µl of BlueMix Lysis Buffer into 200 µl of PD1 Buffer.
3. Transfer the mixture to the 1.5 ml microcentrifuge tube containing the cell pellet. Resuspend the cell pellet completely by vortex or pipette. Continue to vortex or pipette until all traces of the cell pellet have been dissolved.

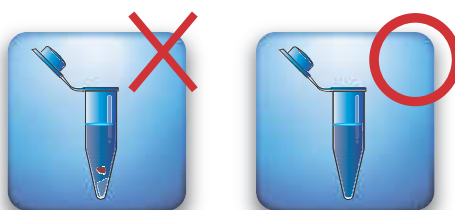
6

Lysis

4. Add 200 µl of PD2 Buffer and mix gently by inverting the tube 10 times. **Do not vortex** to avoid shearing genomic DNA.
5. Close PD2 Buffer bottle immediately after use to avoid CO₂ acidification.
6. Let stand at room temperature for at least 2 minutes to ensure the lysate is homogeneous. **Do not exceed 5 minutes.**

Notes while using BlueMix Lysis Buffer

After adding PD2 Buffer, any precipitates will be completely dissolved and the color of the suspension will become blue. If colorless regions or brownish cell clumps are still visible, continue mixing until the suspension is completely blue.



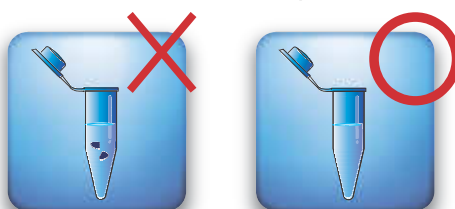
Continue mixing until the suspension is completely blue.

Neutralization

7. Add 300 µl of PD3 Buffer and mix immediately by inverting the tube 10 times. **Do not vortex.**
8. Centrifuge at 14,000-16,000 x g for 3 minutes at room temperature. If using >5 ml of bacterial cells, centrifuge at 16,000-20,000 x g for 5-8 minutes. During centrifugation, place a QPD Column in a 2 ml Collection Tube.

Notes while using BlueMix Lysis Buffer

After adding PD3 Buffer, the suspension turns colorless. If blue regions remain in the suspension, continue mixing until it becomes completely colorless. No traces of blue indicates correct mixing.



Continue mixing until the suspension is completely colorless.

DNA Binding

9. Transfer all of the supernatant to the QPD Column. Use a narrow pipette tip to ensure the supernatant is completely transferred without disrupting the white precipitate. Centrifuge at 14,000-16,000 x g for 30 seconds at room temperature.
10. Discard the flow-through and place the QPD Column back into the 2 ml Collection Tube.

Wash**For Improved Downstream Sequencing Reactions**

Add 400 µl of W1 Buffer into the QPD Column. Centrifuge at 14,000-16,000 x g at room temperature for 30 seconds. Discard the flow-through and place the QPD Column back into the 2 ml Collection Tube. Proceed with Wash Buffer addition.

W1 Buffer is essential for efficient sequencing reactions by removing nuclease contamination and should be added prior to Wash Buffer addition. If you are not performing sequencing reactions, W1 Buffer is not required. Proceed directly to Wash Buffer addition.

For Standard Plasmid DNA Purification

Add 600 µl of Wash Buffer (ethanol added) in the QPD Column. Centrifuge at 14,000-16,000 x g for 30 seconds at room temperature. Discard the flow through then place the QPD Column back in the 2 ml Collection Tube. Centrifuge at 14,000-16,000 x g for 3 minutes at room temperature to dry the column matrix. Transfer the dried QPD Column to a new 1.5 ml microcentrifuge tube.

Perform Wash Buffer steps twice for salt sensitive downstream applications.

DNA Elution

Add 50 µl of Elution Buffer¹, TE² or water³ into the CENTER of the column matrix. Let stand for at least 2 minutes to allow Elution Buffer, TE or water to be completely absorbed. Centrifuge at 14,000-16,000 x g for 2 minutes at room temperature to elute the purified DNA.

¹If a higher DNA concentration is required, use 30 µl of Elution Buffer (10 mM Tris-HCl, pH8.5) then repeat the Elution step by adding the same 30 µl of Elution Buffer (which now contains the eluted DNA) to the center of the column matrix again. If maximum DNA yield is required, use 100 µl of Elution Buffer (DNA concentration will be diluted). Ensure that Elution Buffer is added into the center of the QPD Column matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated Elution Buffer (60~70°C).

²Using TE is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Check elution notes listed in page 4 for more details.

³If using water for elution, check elution notes listed in page 4 for more details.

8

5

Troubleshooting

Problem	Possible Reasons / Solution
Low yield	Bacterial cells were not lysed completely Too many bacterial cells were used. If more than 10 A ₆₀₀ units of bacterial cultures are used, separate them into multiple tubes. Following PD3 Buffer addition, break up the precipitate by inverting or pipetting to ensure higher yield.
	Incorrect Wash Buffer Ensure Ethanol was added to Wash Buffer prior to use.
	Incorrect DNA Elution Step Ensure Elution Buffer was added into the center of QPD Column matrix and was completely absorbed.
Eluted DNA does not perform well in downstream applications	Incomplete DNA Elution If plasmid DNA is larger than 10 kb, use preheated Elution Buffer (60-70°C) in Elution Step to improve the elution efficiency.
	Residual ethanol contamination After wash step, dry QPD Column with additional centrifugation at top speed for 5 minutes or incubation at 60°C for 5 minutes.
	RNA contamination Prior to using PD1 Buffer, ensure that RNase A was added. If RNase A added PD1 Buffer is out of date, add additional RNase A. Too many bacterial cells were used, reduce sample volume.
	Genomic DNA contamination Do not use overgrown bacterial culture. Use only fresh cultures as they will contain less genomic DNA than old cultures. During PD2 and PD3 Buffer addition, mix gently to prevent genomic DNA shearing.
	Nuclease contamination If host cells have high nuclease activity (e.g., endA ⁻ strains), perform following wash steps to remove residual nuclease. After DNA Binding Step, add 400 ul of W1 Buffer into QPD Column. Centrifuge the QPD Column at 14,000-16,000 x g for 30 seconds at room temperature. Then proceed with Wash Buffer addition.

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Notes

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