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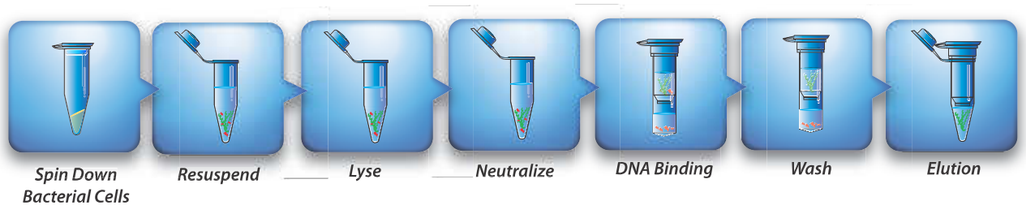
Real Genomics™

Store at room temperature 15°C~25°C



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HiYield™ Plasmid Mini Kit Protocol Book

High Yield Plasmid DNA from 1-5 ml of Bacterial Cultures

Cat. No. **YPD100 / YPD300**

Real Genomics™

Index

HiYield™ Plasmid Mini Kit

HiYield™ Plasmid Mini Kit

Store at room temperature 15°C~25°C

Cat. No. **YPD100**	Cat. No. **YPD300**
100 mini preps / kit	300 mini preps / kit
PD1 Buffer: 25 ml *	PD1 Buffer: 65 ml*
PD2 Buffer: 25 ml **	PD2 Buffer: 75 ml **
PD3 Buffer: 45 ml	PD3 Buffer: 100 ml
W1 Buffer : 45 ml	W1 Buffer :130 ml
Wash Buffer (concentrated): 25 ml ***	Wash Buffer (concentrated): 50 ml ***
Elution Buffer: 6 ml	Elution Buffer: 30 ml
RNase A: 50 µl	RNase A: 130 µl
PD Column: 100 pcs	PD Column: 300 pcs
2 ml Collection Tube: 100 pcs	2 ml Collection Tube: 300 pcs

Sample: 1-5 ml of Bacterial Cultures
Yield: Up to 30 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, 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.

Description

HiYield™ Plasmid Mini Kit is designed for rapid isolation of up to 30 µg of plasmid DNA from 1-5 ml of bacterial cultures without phenol extraction and alcohol precipitation. The entire procedure can be completed within 15 minutes. Typical yields are 20-30 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 FastIon™ Plasmid Midi/Maxi Kits or FastIon Plus™ Plasmid Midi Kits.

Features

Purification of up to 30 µg of pure plasmid DNA within 15 minutes.
High, reproducible recovery of plasmid DNA.
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™ 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™ Plasmid Mini Kits should be stored dry at room temperature (15–25°C) for up to 24 months without showing any reduction in performance and quality. After addition of RNase A, 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.

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, 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.

Centrifugation Notes

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

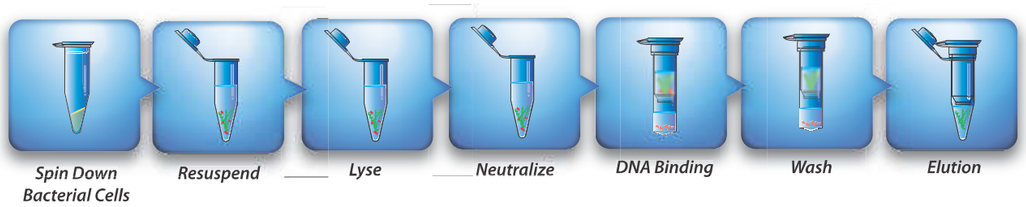
Elution Notes

1. Ensure that the elution buffer, TE or water is dispensed directly onto the center of the PD 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. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the PD Column matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated water (60~70°C).

Protocol

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

This protocol is designed for preparation of up to 30 µg of high-copy plasmid or 10 µg of low-copy plasmid using HiYield™ Plasmid Mini Kit. The typical yield is about 20-30 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.



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, 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 10^9 E. coli grown in LB medium) to a 1.5 ml microcentrifuge tube.
2. Centrifuge at 14,000-16,000 x g for 1 minute at room temperature to form a cell pellet. Then discard the supernatant completely.
3. Use a narrow pipette tip to ensure the supernatant is completely removed. Repeat the harvesting step as required.
4. If more than 1.5 ml of cells are used, repeat the harvesting step. For samples volume between 1.5-5 ml, use the same 1.5 ml microcentrifuge tube.

Resuspension

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

Lysis

7. Add 200 µl of PD2 Buffer (make sure any precipitates are dissolved) and mix gently by inverting the tube 10 times. Do not vortex to avoid shearing genomic DNA.
8. Close PD2 Buffer bottle immediately after use to avoid CO_2 acidification.
9. Let stand at room temperature for at least 2 minutes (Do not exceed 5 minutes) to ensure the lysate is homogeneous.

Neutralization

10. Add 300 µl of PD3 Buffer and mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing genomic DNA.
11. Centrifuge at 14,000-16,000 x g for 3 minutes at room temperature.
12. During centrifugation, place a PD Column in a 2 ml Collection Tube.

DNA Binding

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

HiYield™ Plasmid Mini Kit

Wash

For Improved Downstream Sequencing Reactions

16. Add 400 µl of W1 Buffer into the PD Column.
17. Centrifuge at 14,000-16,000 x g at room temperature for 30 seconds.
18. Discard the flow-through and place the PD Column back into the 2 ml Collection Tube.
19. 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

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

Perform Wash Buffer steps twice for salt sensitive downstream applications.

DNA Elution

If a higher DNA concentration is required, use 30 µl of Elution Buffer.
If maximum DNA yield is required, use 100 µl of Elution Buffer (DNA concentration will be diluted).

20. Add 50 µl of Elution Buffer[1], TE[2] or water[3] into the CENTER of the column matrix.
21. Let stand for at least 2 minutes to allow Elution Buffer, TE or water to be completely absorbed.
22. Centrifuge at 14,000-16,000 x g for 2 minutes at room temperature to elute the purified DNA.

[1] If 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 PD Column matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated Elution Buffer (60~70°C).

[2] Using 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.

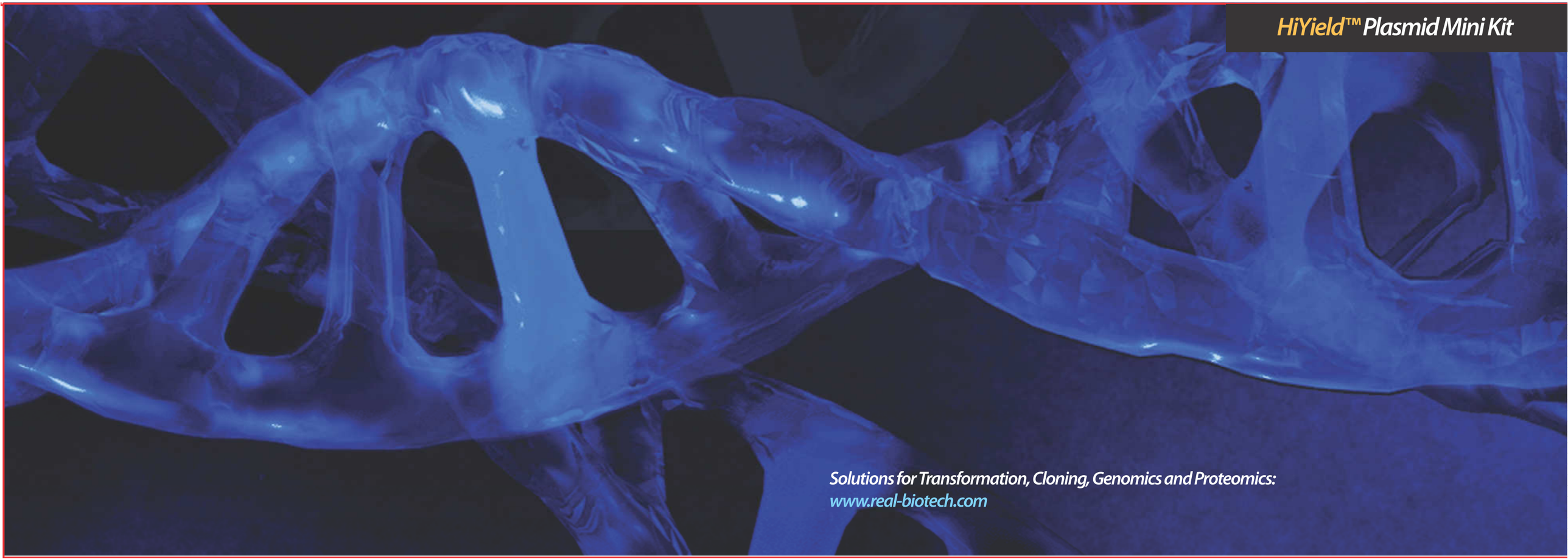
[3] If using water for elution, check elution notes listed in page 4 for more details.

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 PD Column matrix and was completely absorbed.
	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.
Eluted DNA does not perform well in downstream applications	**Residual ethanol contamination** After wash step, dry PD 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 PD Column. Centrifuge the PD Column at 14,000-16,000 x g for 30 seconds at room temperature . Then proceed with Wash Buffer addition.

Notes

HiYield™ Plasmid Mini Kit



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