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HiYield™ Plasmid Large DNA Mini Kit

Cat. No.:	YPL100	YPL300
Product Name:	HiYield™ Plasmid Large DNA Mini Kit	
Reactions:	100	300
Sample:	Up to 30 µg of plasmid/cosmid DNA	
DNA Size Range:	10-50 Kb	
Format:	Spin column	
Operation:	Centrifuge	
Operation Time:	Within 15 minutes	
Elution Volume:	50-100 µl	

Introduction

HiYield™ Plasmid Large DNA Mini Kit is designed for rapid isolation of up to 30 µg of 10-50 kb plasmid or cosmid DNA from 1-4 ml of bacterial cultures within 15 minutes without phenol extraction and alcohol precipitation. Typical yields are 20-30 µg for high-copy plasmid or 3-10 µg for low-copy plasmid from 4ml of bacterial cultures. Purified DNA is ready for direct use in DNA Sequencing, DNA Library Screening and Analysis, Restriction Enzyme Digestion, PCR, Ligation and Transformation.

For large scale plasmid purification, we recommend HiYield™ Plasmid Midi Kit and ion exchange based FastIon™ Plasmid Midi/Maxi Kits.

Features

Purify 10-50 kb plasmid DNA within 15 minutes.
High, reproducible recovery of plasmid DNA.
Rapid and simple procedure.
No phenol, chloroform or alcohol.

Applications

Purified DNA is ready for direct use in DNA Sequencing, DNA Library Screening and Analysis, Restriction Enzyme Digestion, PCR, Ligation and Transformation.

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Contents

ITEM	YPL100	YPL300
PL1 Buffer*	25 ml	65 ml
PL2 Buffer**	25 ml	75 ml
PL3 Buffer	45 ml	100 ml
W1 Buffer	45 ml	130 ml
Wash Buffer (concentrated)	25 ml	50 ml
Elution Buffer	6 ml	30 ml
RNase A (50 µg/µl)	50 µl	130 µl
PL Column	100 pcs	300 pcs
2 ml Collection Tube	100 pcs	300 pcs

*Add provided RNase A to the PL1 Buffer and store at 4ºC (RNase A was already added to YPL10S samples).

**If precipitates have formed in the PL2 Buffer, warm the buffer in a 37ºC water bath, followed by gentle shaking to dissolve

*** Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds prior to initial use. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Caution

PL3 Buffer and W1 Buffer contain guanidine hydrochloride. During the procedure, always wear a lab coat, disposable gloves and protective goggles.

Quality Control

The quality of HiYield™ Plasmid Large DNA Mini Kits are tested on a lot-to-lot basis by isolation of plasmid DNA from 4 ml overnight culture of E.coli (DH10B) transformed with the plasmid pBluescript (A600 > 2 units/ml). Purified DNA is quantified with a spectrophotometer and the yield of plasmid DNA is more than 20µg with A260/A280 ratio 1.7 to 1.9. Then 1 µg of the purified DNA is used in Eco R1 digestion and checked by electrophoresis.

References

(1) Birnboim, H. C., and Doly, J. (1979) Nucleic Acids Res. 7, 1513

(2) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615

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Protocol

Please read the entire instruction manual prior to starting.

Caution: During operation, always wear a lab coat, disposable gloves, and protective goggles.

Things to Do before Starting:

1. Add provided RNase A to the PL1 Buffer and store at 4ºC (RNase A was already added to YPL10S samples).
2. If precipitates have formed in the PL2 Buffer, warm the buffer in a 37ºC water bath, followed by gentle shaking to dissolve
3. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds prior to initial use. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Additional Requirements: 1.5ml microcentrifuge tubes, absolute ethanol.

Harvesting	★Transfer 1.5 ml of cultured bacterial cells to a microcentrifuge tube. ★Centrifuge at 14,000-16,000 x g for 1 minute then discard the supernatant. ★If more than 1.5 ml of cultured bacterial cells is used, repeat the Harvesting step.
Step 1 **Resuspension**	★Add 200 µl of PL1 Buffer (make sure RNase A was added). ★Resuspend the cell pellet by vortex or pipetting.
Step 2 **Lysis**	★Add 200 µl of PL2 Buffer then mix gently by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. ★Let stand at room temperature for at least 2 minutes to ensure the lysate is homogeneous. At this time, pre-heat the required Elution Buffer to 70ºC (for Step 6 DNA Elution).
Step 3 **Neutralization**	★Add 300 µl of PL3 Buffer and mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. ★Centrifuge at 14,000-16,000 x g for 3 minutes.
Step 4 **DNA Binding**	★Place a PL Column in a 2 ml Collection Tube. ★ Add the supernatant from Step 3 to the PL Column then centrifuge at 14,000-16,000 x g for 30 seconds. ★Discard the flow-through then place the PL Column back in the 2 ml Collection Tube.

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Step 5 Wash	★Add 400 µl of W1 Buffer into the PL Column then centrifuge at 14,000-16,000 x g for 30 seconds. ★ Discard the flow-through then place the PL Column back in the 2 ml Collection Tube. ★ Add 600 µl of Wash Buffer (make sure ethanol was added) into the PL Column. ★ Centrifuge at 14,000-16,000 x g for 30 seconds. ★ Discard the flow through then place the PL Column back in the 2 ml Collection Tube. ★ Centrifuge at 14,000-16,000 x g again for 3 minutes to dry the column matrix.
Step 6 DNA Elution	★ Transfer the dried PL Column to a new microcentrifuge tube. ★ Add 50 µl of pre-heated Elution Buffer or TE into the center of the column matrix. ★ Let stand for at least 2 minutes to ensure the Elution Buffer or TE is absorbed by the matrix. ★ Centrifuge at 14,000-16,000 x g for 2 minutes to elute the purified plasmid DNA.

Troubleshooting

Problem	Possible Reasons/ Solution
Low yield	**Bacterial cells were not lysed completely** ★If more than 10 OD_{600} units of bacterial culture are used, dilute into multiple tubes. ★Following PL3 Buffer addition, pipetting or inverting will help to ensure the sample is homogeneous.
	Incorrect DNA Elution Step ★Ensure that Elution Buffer is added into the center of the PL Column matrix and is completely absorbed.
Purified DNA does not perform well in downstream applications	**Residual ethanol contamination** ★Following the Wash step, dry the PL Column with additional centrifugation at 14,000-16,000 x g for 5 minutes.
	RNA contamination ★ Prior to using PL1 Buffer, be sure RNase A is added.
	Genomic DNA contamination ★ Do not use overgrown bacterial cultures. ★ During PL2 and PL3 Buffer addition, mix gently to prevent genomic DNA shearing.
	Nuclease contamination ★ Following the DNA Binding step, add 400 µl of W1 Buffer into the PL Column and incubate for 2 minutes at room temperature. Centrifuge the PL Column at 14,000-16,000 x g for 30 seconds and proceed with the standard wash step.

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