96 Plasmid Purification Kits

NucleoGen
BIOTECHNOLOGY

Contents

Kit Contents

	4 x 96 Plates	25 x 96 Plates	50 x 96 Plates
Catalog No.	**4304**	**4325**	**2 x 4325**
Number of preparations	4 x 96	25 x 96	50 x 96
96 Clarification plates	4	25	50
96 Binding plates	4	25	50
96 Elution plates	4	25	50
Cell Resuspension Solution	+	+	+
Cell Lysis Solution	+	+	+
Neutralization Solution*	+	+	+
Wash A Solution (concentrate)	+	+	+
Elution Buffer	+	+	+
RNase A†	+	+	+
Tape	+	+	+

* Neutralization Solution contain chaotropic salts which are irritants and not compatible with disinfecting agents containing bleach. Take appropriate laboratory safety measures and wear gloves when handling.
† Provided as a 100 mg/ml solution.

Principle

The Nucleogen 96 Plasmid Purification Kits procedure is based on alkaline lysis of bacterial cells followed by adsorption of DNA onto silica in the presence of high salt.

The procedure consists of three basic steps:
- preparation and clearing of a bacterial lysate
- adsorption of DNA onto the 96 Plasmid Purification membrane
- washing and elution of plasmid DNA

All steps are performed without the use of phenol, chloroform, CsCl, ethidium bromide, and without alcohol precipitation.

Alkaline lysis of bacteria

The 96 Plasmid Purification procedure uses the modified alkaline lysis method of Birnboim and Doly. Bacteria are lysed under alkaline conditions, and the lysate is subsequently neutralized and adjusted to high-salt binding conditions in one step, ready for purification on the membrane.

Lysate clearing

Following alkaline lysis of bacterial cultures, the crude lysates are loaded directly onto the 96 Clarification plate. Denatured and precipitated cellular components are removed by filtration through the 96 Clarification plate.
In the 96 Plasmid Purification procedures, lysates are cleared by centrifugation or vacuum manifold.

DNA adsorption to the Plasmid Purification membrane

96 Binding plates use a silica-gel membrane for selective adsorption of plasmid DNA in high-salt buffer and elution in low-salt buffer. The optimized buffers in the lysis procedure combined with the unique membrane ensure that only DNA will be adsorbed, while RNA, cellular proteins, and metabolites are not retained on the membrane but are found in the flow-through.

Washing and elution of plasmid DNA

Salts are efficiently removed by a brief wash step with Wash A Solution. High-quality plasmid DNA is then eluted from the 96 Binding plate with Elution Buffer or Water. The purified DNA is ready for immediate use in a range of applications — no need to precipitate, concentrate, or desalt.
Note: Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water for elution, make sure that the pH value is within this range. Store DNA at –20°C when eluted with water since DNA may degrade in the absence of a buffering agent.

DNA yield

Plasmid yield with the 96 Plasmid Purification system varies depending on plasmid copy number per cell, the individual insert in a plasmid, factors that affect growth of the bacterial culture, the elution volume, and the elution incubation time. A 1 ml overnight culture can yield from 3 to 4 ug of plasmid DNA. To obtain the optimum combination of DNA quality and concentration, eluting plasmid DNA in a volume of 50 ul, and performing a short incubation after addition of the elution buffer.

Important Notes

Please read the following notes before starting any of the 96 Plasmid Purification procedures.

Before equipment

- **Add the RNase A Solution to Cell Resuspension Solution**, mix, store at 2-8℃
- Centrifuge table tap (max. speed 6,000 rpm or 5788 x g)
- Check lysis, Neutralization Solution before use for salt precipitation. If any precipitated, heat to dissolve (37℃). Wear, gloves when handling these buffer.
- **Add ethanol (95-100%) to Wash A Solution**, before use (see bottle label for volume).
- **96 Deep Well Plates (not include)**

96 Plasmid Purification Kit Protocol
Using a Vacuum Manifold

Please read "Important Notes" on pages 4 before starting.

Vacuum notes:
- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during manipulations.
- Wear safety glasses when working near a manifold under pressure.
- The vacuum pressure is the pressure differential between the inside of the manifold and the atmosphere (standard atmospheric pressure: 1013 mmbar or 760 mmHg) and can be measured using a vacuum regulator.
- Use of a vacuum pressure lower than recommended may reduce DNA yield and purity.

1. **Prepare E.coli into 1 ml aliquots of LB or 2X LB containing appropriate antibiotic on sterile 96 deep well plate (2 ml capacity, not include). Cover plates with lid. Incubate at 37ºC at 250-320 rpm for 20 to 24 hours (O.D.650 ≈ 1.3).**

2. **Centrifuge at 1500 x g for 10 min. After centrifugation, immediately decant culture supernatant to a container for proper disposal.** Invert and tap the plates firmly on several layers of paper towels on the bench to remove residual culture supernatant.

3. **Resuspend pelleted bacterial cells in 100 ul Cell Resuspension Solution using a vortex or a plate shaker, and than mix by pippetting. Complets resuspension is important.**

4. **Add 100 ul Cell Lysis Solution. Mix immediately and vigorously (maximum speed) with a plate shaker or vortex for 1 min. Incubate for an additional 2 min at room temperature. Do not excess lysate to Cell Lysis Solution for more than 5 min or plasmid maybe denature irreversibly.**

5. **Add 170 ul Neutralization Solution. Mix immediately and vigorously (maximum speed) with a plate shaker for 2 min.**

6. **To remove the lysate, lower the piptte tips down the sides of the deep wells through the lysates until reaching bottom. Slowly remove 175 ul of lysates from the bottom of each deep well, and dispense into the corresponding well of 96 Clarification plate. Entering the same well a second time, remove 175 ul from the deep well plate, and transfer it to the corresponding wells of 96 Clarification plate.**

7. **Place the 96 Binding plate in the bottom of the vacuum manifold** (Millipore: MAVM096 or equivalent).

8. **Place the 96 Clarification plate on top of the manifold, and adjust the vacuum to 250 mmbar. Do not excess 250 mmbar vacuum setting during filtration of the lysate to ensure uniform filtration.**

9. **Apply the vacuum for 5-10 min, drawing the lysate through the 96 Clarification plate into a 96 Binding plate. Discard a 96 Clarification plate.**

10. **Remove the 96 Binding plate from inside the manifold, and place a 96 Binding plate on top of the empty manifold. Apply full vacuum for 1-2 min.** Plasmid DNA is now bound to the 96 Binding plate.

11. **Add 300 ul of Wash A Solution to each well of the 96 Binding plate. Apply full vacuum for 1 min.**

12. **Repeat step 11, but apply vacuum for 3 min.**

13. **Remove the 96 Binding plate from the manifold. Tap the plates firmly on several layers of paper towels on the bench to remove residual alcohol.**

14. **Place the 96 Binding plate on top of deep well plate (not include) and centrifuge at 1000 x g for 10min to dry (Alternatively, the plate can be dry at 60°C for 30 min).**

15. **To elute the plasmid DNA, place the a 96 Binding plate on top of a Elution plate and add Elution Buffer (10 mM Tris, pH 8.5) or nuclease-free water to the center of each well of the 96 Binding plate, let stand for 1 min.**
    This protocol consistently delivers 3-4 ug of pGem 3Zf plasmid DNA (at 70-90 ng/ul) from E.coli JM109. Yields will vary depending of replicon and insert. If higher concentration are required, dissolve in 50 ul of Elution Buffer (10 mM Tris, pH 8.5) or nuclease-free water instead of 70 ul in step 15.

16. **Centrifuge at 1000 x g for 5 min (eluted volume is typically 45-50 ul), or elute by vacuum.**

96 Plasmid Purification Kit Protocol
Using a Centrifuge

Please read "Important Notes" on pages 4 before starting.

1. **Inoculate E.coli into 1 ml aliquots of LB or 2X LB containing appropriate Antibiotic on sterile 96 deep well plate (2 ml capacity, not include). Cover plates with lid. Incubate at 37ºC at 250-320 rpm for 20 to 24 hours (O.D.650 ≈1.3).**

2. **Centrifuge at 1500 x g for 10 min. After centrifugation, immediately decant culture supernatant to a container for proper disposal.** Invert and tap the plates firmly on several layers of paper towels on the bench to remove residual culture supernatant.

3. **Resuspend pellets in 100 ul of Cell Resuspension Solution using a vortex or a Plate shaker, and than mix by pippetting. Complete reouspension is important.**

4. **Add 100 ul Cell Lysis Solution. Mix immediately and vigorously (maximum speed) with a plate shaker or vortex for 1 min. Incubate for an additional 2 min at room temperature. Do not excess lysate to Cell Lysis Solution for more than 5 min or plasmid maybe denature irreversibly.**

5. **Add 170 ul Neutralization Solution. Mix immediately and vigorously (maximum speed) with a plate shaker for 2 min.**

6. **To remove the lysate, lower the piptte tips down the sides of the deep wells through the lysates until reaching bottom. Slowly remove 175 ul of lysates from the bottom of each deep well, and dispense into the corresponding well of 96 Clarification plate. Entering the same well a second time, remove 175 ul from the deep well plate, and transfer it to the corresponding wells of 96 Clarification plate.**

7. **Place the 96 Clarification plate on top of a 96 Binding plate.**

8. **Place the set prepared in step 7 on top of a deep well plate (not include).**

9. **Centrifuge at 2000 x g for 20 min. Discard the 96 Clarification plate. And the waste in deep well plate (not include).**

10. **Place the 96 Binding plate on top of a deep well plate (not include).**

11. **Add 300 ul of Wash A Solution to each well of the 96 Binding plate.**

12. **Centrifuge at 2000 x g for 5 min. Discard the waste in deep well plate (not include).**

13. **Repeat step 10 and 11, but centrifuge for 10 min. Remove the 96 Binding plate from deep well plate (not include). Place the 96 Binding plate on top of Elution plate.**

14. **To elute the plasmid DNA, add Elution Buffer (10 mM Tris, pH 8.5) or nuclease-free water to the center of each well of the 96 Binding plate, let stand for 1 min. This protocol consistently delivers 3-4 ug of pGem 3Zf plasmid DNA (at 70-90 ng/ul) from E.coli JM109. Yields will vary depending on replicon and insert. If higher concentration are required, dissolve in 50 ul of Elution Buffer (10 mM Tris, pH 8.0) or nuclease-free water instead of 70 ul in step 15.**

15. **Centrifuge at 1000 x g for 5 min (eluted volume is typically 45-50 ul), or elute by vacuum.**

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise.

	Comments and suggestions
Low or no yield	
General	Low yields may be caused by a number of factors. To find the source of the problem, analyze fractions saved from each step in the procedure on an agarose gel. A small amount of the cleared lysate and the entire flow-through can be precipitated by adding 0.7 volumes isopropanol and centrifuging at maximum speed for 30 minutes. The entire wash flow-through can be precipitated by adding 0.1 volumes of 3 M sodium acetate, pH 5.0, and 0.7 volumes of isopropanol.
No DNA in the cleared lysate before loading	
a) Lysate prepared Incorrectly	Check storage conditions and age of buffers.
b) Cell Lysis Solution precipitated	Redissolve by warming to 37°C.
c) Cell resuspension incomplete	Pelleted cells should be completely resuspended in Cell Resuspension Solution. Do not add Cell Lysis Solution until an even suspension is obtained.
DNA is found in the flow-through of cleared lysate	
a) Plasmid Purification membrane overloaded	If rich culture media, such as TB or 2x YT are used, culture volumes must be reduced. It may be necessary to adjust LB culture volume if the plasmid and host strain show extremely high copy number or growth rates.
b) RNase A digestion omitted	Ensure that RNase A is added to Cell Resuspension Solution before use.
c) RNase A digestion insufficient	Reduce culture volume if necessary. If Cell Resuspension Solution containing RNase A is more than 6 months old, add additional RNase A.
DNA is found in the wash flow-through	
a) Ethanol omitted from wash buffer	Repeat procedure with correctly prepared wash buffer (Wash A Solution).
Little or no DNA in eluate	
a) Elution buffer incorrect	DNA is eluted only in the presence of low-salt buffer, e.g., Elution Buffer (10 mM Tris·Cl, pH 8.5) or Water. Elution efficiency is dependent on pH. The maximum efficiency is achieved between pH 7.0 and 8.5. When using water for elution, make sure that the pH value is within this range.
b) Elution buffer incorrectly dispensed onto membrane	Add elution buffer to the center of the Plasmid Purification membrane to ensure that the buffer completely covers the surface of the membrane for maximum elution efficiency.
Low DNA quality **DNA does not perform well**	
a) Eluate salt concentration too high	For the 96 Binding plate, modify the wash step by incubating the column for 5 minutes at room temperature after adding 300 ul Wash A Solution and then centrifuging. Ensure that two wash steps are carried out prior to elution.

Comments and suggestions

RNA in the eluate	
a) RNase A digestion omitted	Ensure that RNase A is added to Cell Resuspension Solution before use.
b) RNase A digestion insufficient	Reduce culture volume if necessary. If Cell Resuspension Solution containing RNase A is more than 6 months old, add additional RNase A.
Genomic DNA in the eluate	
a) Cell Lysis Solution added incorrectly	The lysate must be handled gently after addition of Cell Lysis Solution to prevent shearing. Reduce culture volume if lysate is too viscous for gentle mixing.
b) Neutralization Solution added incorrectly	Upon addition of Neutralization Solution, mix immediately but gently.
c) Lysis too long	Lysis must not exceed 5 minutes.
d) Culture overgrown	Overgrown cultures contain lysed cells and degraded DNA. Do not grow cultures for longer than 12–16 hours.

Ordering Information

Products		Contents	Cat. No.
RNA Stabilization Reagent		Tube (50 x 1.5 ml)	3502
		Tube (20 x 5 ml)	3205
		100 ml	3100
		250 ml	3250
Plasmid Purification Mini Kit			
	(200) for negative strain	200 preps	5112
	(200) for positive strain	200 preps	7112
Plasmid Purification Midi Kit	(10)	10 preps	6101
	(50)	50 preps	6105
	(100)	100 preps	6110
Plasmid Purification Maxi Kit	(6)	6 preps	7106
	(24)	24 preps	7124
	(50)	50 preps	7150
Gel Extraction Kit	(50)	50 preps	5215
	(200)	200 preps	5212
Highcon Gel Extraction Kit	(50)	50 preps	2215
	(200)	200 preps	2212
Bead Type (NaI) Gel Extraction Kit	(200)	200 preps	1232
	(400)	400 preps	1234
	(600)	600 preps	1236
PCR Purification Kits	(50)	50 preps	5315
	(200)	200 preps	5312
Highcon PCR Purification Kit	(50)	50 preps	2315
	(200)	200 preps	2312
DNA Clean-up Kits	(50)	50 preps	1415
	(200)	200 preps	1412
Genomic Blood Spin Mini Kit	(50)	50 preps	1515
	(200)	200 preps	1512
Genomic Blood Spin Midi Kit	(20)	20 preps	6520
	(50)	50 preps	6550
	(100)	100 preps	6500
Genomic Blood Spin Maxi Kit	(6)	6 preps	7506
	(24)	24 preps	7524
	(50)	50 preps	7550

Ordering Information

Products		Contents	Cat. No.
Genomic Cell / Tissue Spin Mini Kit	(50)	50 preps	1545
	(200)	200 preps	1542
Genomic Cell / Tissue Spin Midi Kit	(20)	20 preps	
	(50)	50 preps	
	(100)	100 preps	
Genomic DNA Isolation, Flexible		100 Isolation	1521
		500 Isolation	1525
		10 ml x 100 Isolation	
Apoptotic DNA Ladder Kit		50 preps	2505
96 PCR Purification Kit			
4 x 96 plates(binding, elution), buffer, tape			4304
25 x 96 plates(binding, elution), buffer, tape			4325
50 x 96 plates(binding, elution), buffer, tape			2 x 4325
96 Plasmid Purification Kit			
4 x 96 plates(clarification, binding, elution), buffer, tape			4104
25 x 96 plates(clarification, binding, elution), buffer, tape			4125
96 Genomic Blood Spin Kit			
4 x 96 plates(binding, elution), buffer, tape			
25 x 96 plates(binding, elution), buffer, tape			
96 Genomic Cell / Tissue Spin Kit			
4 x 96 plates(binding, elution), buffer, tape			
25 x 96 plates(binding, elution), buffer, tape			
50 x 96 plates(binding, elution), buffer, tape			2 x

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