Plasmid Purification Kits

☐ **Plasmid Purification Mini Kit**

☐ **Plasmid Purification Midi Kit**

☐ **Plasmid Purification Maxi Kit**

NucleoGen
BIOTECHNOLOGY

Contents

Kit Contents

	Mini Kits		Midi Kits			Maxi Kits		
Catalog No.	**5112 / 7112**		**6101 / 6105 / 6110**			**7106 / 7124 / 7150**		
Number of preparations	200	200	10	50	100	6	24	50
Nucleogen Spin Columns	200	200	10	50	100	6	24	50
Collection tubes (2 ml)	200	200	10	50	100	6	24	50
Cell Resuspension Solution	+	+	+	+	+	+	+	+
Cell Lysis Solution	+	+	+	+	+	+	+	+
Neutralization Solution*	+	+	+	+	+	+	+	+
Wash A Solution (concentrate)	+	+	+	+	+	+	+	+
Wash B Solution*	–	+	–	–	–	–	–	–
Elution Buffer	+	+	+	+	+	+	+	+
RNase A†	+	+	+	+	+	+	+	+

* Neutralization Solution and Wash B 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 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 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 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 Plasmid Purification Kit. Denatured and precipitated cellular components are removed by filtration through the membrane. Particle-free filtrates flow directly into the wells of the Plasmid Purification module.
In the Plasmid Purification procedures, lysates are cleared by centrifugation.

DNA adsorption to the Plasmid Purification membrane

Plasmid Purification columns 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

Endonucleases are efficiently removed by a brief wash step with Wash B Solution. This step is essential when working with *endA*+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, to ensure that plasmid DNA is not degraded. The Wash B solution step is also necessary when purifying low-copy plasmids, where large culture volumes are used.
Salts are efficiently removed by a brief wash step with Wash A Solution. High-quality plasmid DNA is then eluted from the spin column 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 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 1.5 ml overnight culture can yield from 5 to 15 ug of plasmid DNA (Mini). 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 (Mini).

Important Notes

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

Before equipment

- **Add the RNase A Solution to Cell Resuspension Solution**, mix, store at 2-8℃
- Microcentrifuge table tap (max. speed ≥10,000 x g or ~14,000 rpm) or Centrifuge table tap (max. speed 6,000 rpm)
- 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).

Plasmid Purification Mini Kit Protocol
Using a Microcentrifuge

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

1. **Prepare a 1-5 ml (low-copy; 5-10 ml) culture of plasmid-containing E.coli in LB broth which contains the appropriate antibiotic (e.g., 50 ug/ml ampicillin) and grow the cells with shaking at 37℃ overnight.**

2. **Resuspend pelleted bacterial cells in 250 ul Cell Resuspension Solution and transfer to a microcentrifuge tube.**

3. **To the tube, add 250 ul Cell Lysis Solution and mix the contains of the tube gently by inversion (5 times). (Do not vortex). Incubate until the cell suspension clears, approximately 1-5 minutes.**

4. **Add 350 ul Neutralization Solution and inverte the tube immediatly but gently 5 times. (do not vortex).** A white precipitate will form.

5. **Centrifuge the suspension in a microcentrifuge for 10 min at RT.**
   Transfer the plasmid-containing supernatant to the Mini-column by pipetting.

6. **Centrifuge for 1 min. Discard the flow-through.**

7. **(Optional) : Wash the Mini-column by adding 500 ul Wash B Solution and centrifuging for 1 min. Discard the flow-through.**
   This step is necessary to remove trace nuclease activity when using end*A*+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α do not require this additional wash step.

8. **Wash the Mini-Column by adding 750 ul Wash A Solution and centrifuging for 1 min. Discard the flow-through.**

9. (Optional) Wash the Mini-column by adding 400 ul Wash A Solution and centrifuging for 3 min. Discard the flow-through.

10. **Discard the flow-through, and centrifuge for an add 2 min to remove residual Wash A Solution.**

11. **Place the Mini-column in clean 1.5 ml microcentrifuge tube.**
    To elute DNA, add 50 ul Elution Buffer (10 mM Tris·Cl, pH 8.5) or Water to the center of each the Mini-column, let stand for 1 min, and centrifuge for 3 min.

 # The type of E.coli strains

***EndA*+ strains** : JM83, JM101, JM110, BL21(DE3), CJ236, LE392, MC1061, NM series, P2392, PR series, RR1, TB1, TG1, BMH71-18, ES1301, wild-type, and etc.

***EndA*− strains** : DH5α, DH1, 5, 20, 21, MM294, SK1590, SRB, XL1-Blue, XLO, and etc.

Plasmid Purification Mini 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 millibar 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. **Resuspend pelleted bacterial cells in 250 ul Cell Resuspension Solution and transfer to a microcentrifuge tube.**
   Ensure that RNase A has been added to Cell Resuspension Solution. No cell clumps should be visible after resuspension of the pellet.

2. **Add 250 ul Cell Lysis Solution and invert the tube gently 4-6 times to mix.**
   Do not vortex, as this will result in shearing of genomic DNA.
   If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

3. **Add 350 ul Neutralization Solution and invert the tube immediately but gently 4-6 times.**
   To avoid localized precipitation, immediately after addition of Neutralization solution mix the solution gently but thoroughly. The solution should become cloudy.

4. **Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.**
   A compact white pellet will form.
   During centrifugation, prepare the vacuum manifold and Mini-columns:
   - Insert each Mini-column into a luer connector on the luer adapter(s) in the vacuum manifold.

5. **Apply the supernatant from step 4 to the Mini-column by decanting or pipetting.**

6. **Switch on vacuum source to draw the solution through the Mini-columns, and then switch off vacuum source.**

7. **(Optional): Wash the Mini-column by adding 500 ul Wash B Solution. Switch on vacuum source. After the solution has moved through the Mini-column, switch off vacuum source.**
   This step is necessary to remove trace nuclease activity when using endA+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α do not require this additional wash step.

8. **Wash the Mini-column by adding 750 ul Wash A Solution. Switch on vacuum source to draw the wash solution through the Mini-column, and then switch off vacuum source.**

9. **Transfer the Mini-columns to a microcentrifuge tube. Centrifuge for 1 min.**
   IMPORTANT: This extra spin is necessary to remove residual Wash A Solution. Residual ethanol from Wash A Solution may inhibit subsequent enzymatic reactions.

10. **Place the Mini-column in a clean 1.5 ml microcentrifuge tube.**
    To elute DNA, add 50 ul Elution Buffer (10 mM Tris·Cl, pH 8.5) or Water to the center of the Mini-column, let stand for 1 min, and centrifuge for 1 min.

Plasmid Purification Midi Kit Protocol
Using a Centrifuge

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

1. **Prepare a 25-50 ml (low-copy; 50-100 ml) culture of plasmid-containing E.coli in LB broth which contains the appropriate antibiotic and grow the cells with shaking at 37℃ overnight. Centrifuge at 2500-3500 xg for 10 min.**

2. **Resuspend pelleted bacterial cells in 0.4 ml Cell Resuspension Solution and transfer to a centrifuge tube.**

3. **To the tube, add 0.8 ml Cell Lysis solution and mix the contains of the tube gently by inversion (5 times). (do not vortex). Incubate until the cell suspension clears, approximately 1-5 minutes.**

4. **Add 2.4 ml Neutralization solution and inverte the tube immediately but gently 5 times. (do not vortex).** A white precipitate will form.

5. **Centrifuge the suspension in a centrifuge for 10 min at 2500-3500 x g, RT.**
   Transfer 4 ml the plasmid-containing supernatant to the Nucleogen Midi-column by pipetting.

6. **Centrifuge for 3 min at 2500-3500 x g. Discard the flow-through.**
   Warning: Do not expose the liquid flow through to bleach.

7. **(Optional): Wash the Midi-column by adding 2 ml Wash B Solution (not include) and centrifuging for 2 min. Discard the flow-through.**
   This step is necessary to remove trace nuclease activity when using end*A*+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α do not require this additional wash step.

8. **Wash the Midi-column by adding 3 ml Wash A Solution and centrifuging for 3 min at 2500-3500 x g. Discard the flow-through.**

9. (Optional) Wash the Midi-column by adding 2 ml Wash A Solution and centrifuging for 3 min. Discard the flow-through.

10. **Discard the flow-through, and centrifuge for an add 2 min to remove residual Wash A Solution.**

11. **Place the Midi-column in clean 15 ml centrifuge tube.**
    To elute DNA, add 1 ml Elution Buffer (10 mM Tris-Cl, pH 8.5) or Water to the center of each the Midi-column, let stand for 1 min, and centrifuge for 3 min at 2500-3500 x g.

 # The type of E.coli strains

***EndA*+ strains** : JM83, JM101, JM110, BL21(DE3), CJ236, LE392, MC1061, NM series, P2392, PR series, RR1, TB1, TG1, BMH71-18, ES1301, wild-type, and etc.

***EndA*− strains** : DH5α, DH1, 5, 20, 21, MM294, SK1590, SRB, XL1-Blue, XLO, and etc.

Plasmid Purification Midi 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 millibar 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. **Resuspend pelleted bacterial cells in 0.4 ml Cell Resuspension Solution and transfer to a centrifuge tube.**
   Ensure that RNase A has been added to Cell Resuspension Solution. No cell clumps should be visible after resuspension of the pellet.

2. **Add 0.8 ml Cell Lysis Solution and invert the tube gently 4-6 times to mix. Do not vortex, as this will result in shearing of genomic DNA.**
   If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

3. **Add 2.4 ml Neutralization Solution and invert the tube immediately but gently 4-6 times.**
   To avoid localized precipitation, immediately after addition of Neutralization solution mix the solution gently but thoroughly. The solution should become cloudy.

4. **Centrifuge the suspension in a centrifuge for 10 min at 2500-3500 x g, RT.**
   A compact white pellet will form.
   During centrifugation, prepare the vacuum manifold and Midi-columns:
   - Insert each Midi-column into a luer connector on the luer adapter(s) in the vacuum manifold.

5. **Apply the supernatant from step 4 to the Midi-column by decanting or pipetting.**

6. **Switch on vacuum source to draw the solution through the Midi-columns, and then switch off vacuum source.**

7. **(Optional): Wash the Midi-column by adding 2 ml Wash B Solution (not include). Switch on vacuum source. After the solution has moved through the Midi-column, switch off vacuum source.**
   This step is necessary to remove trace nuclease activity when using endA+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α do not require this additional wash step.

8. **Wash the Midi-column by adding 3 ml Wash A Solution. Switch on vacuum source to draw the wash solution through the Midi-column, and then switch off vacuum source.**

9. **Transfer the Midi-columns to a centrifuge tube. Centrifuge for 2 min at 2500-3500 x g, RT.**
   IMPORTANT: This extra spin is necessary to remove residual Wash A Solution. Residual ethanol from Wash A Solution may inhibit subsequent enzymatic reactions.

10. **Place the Midi-column in a clean 15 ml centrifuge tube.**
    To elute DNA, add 1 ml Elution Buffer (10 mM Tris·Cl, pH 8.5) or Water to the center of the Midi-column, let stand for 1 min, and centrifuge for 3 min at 2500-3500 x g.

Plasmid Purification Maxi Kit Protocol
Using a Centrifuge

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

1. **Prepare a 100-250 ml (low-copy; 200-500 ml) culture of plasmid-containing E.coli in LB broth which contains the appropriate antibiotic and grow the cells with shaking at 37℃ overnight. Centrifuge for 15 min at 2500 x g.**

2. **Resuspend pelleted bacterial cells in 5 ml Cell Resuspension Solution and transfer to a centrifuge tube.**

3. **To the tube, add 10 ml Cell Lysis solution and mix the contains of the tube gently by inversion (5 times). (Do not vortex). Incubate until the cell suspension clears, approximately 1-5 minutes.**

4. **Add 30 ml Neutralization solution and invert the tube immediately but gently 5 times. (Do not vortex).** A white precipitate will form.

5. **Centrifuge the suspension in a centrifuge for 10 min at 2500 x g, RT.**
   Transfer 20 ml of the plasmid-containing supernatant to the Nucleogen Maxi-column by pipetting. (Avoid the white precipitate when transferring the supernatant).

6. **Centrifuge for 5 min at >2500 x g.**

7. **Warning:** Do not let this liquid contact bleach. Remove filter unit, discard the liquid contents from tube and then replace the filter unit in the tube.

8. **Add the remaining lysate to the spin filter and repeat step 5.**

9. **Discard the liquid and replace the Maxi-spin column.**

10. **(Optional): Wash the Maxi-column by adding 20 ml Wash B Solution (not include) and centrifuging for 2 min. Discard the flow-through.**
    This step is necessary to remove trace nuclease activity when using end*A*+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α do not require this additional wash step.

11. **Wash the Maxi-Column by adding 20 ml Wash A Solution and centrifuging for 5 min at 2500 x g. Discard the flow-through.**

12. (Optional): Wash the Maxi-column by adding 5 ml Wash A Solution and centrifuging for 5 min at 2500 x g. Discard the flow-through.

13. **Discard the flow-through, and centrifuge for an add 5 min to remove residual Wash A Solution.**

14. **Place the Maxi-column in clean 50 ml centrifuge tube.**
    To elute DNA, add 4 ml Elution Buffer (10 mM Tris-Cl, pH 8.5) or Water to the center of each the Maxi-column, let stand for 1 min, and centrifuge for 5 min at 2500 x g.

 # The type of E.coli strains

***EndA*+ strains** : JM83, JM101, JM110, BL21(DE3), CJ236, LE392, MC1061, NM series, P2392, PR series, RR1, TB1, TG1, BMH71-18, ES1301, wild-type, and etc.

***EndA*− strains** : DH5α, DH1, 5, 20, 21, MM294, SK1590, SRB, XL1-Blue, XLO, and etc.

Plasmid Purification Maxi 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 millibar 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. **Resuspend pelleted bacterial cells in 5 ml Cell Resuspension Solution and transfer to a centrifuge tube.**
   Ensure that RNase A has been added to Cell Resuspension Solution. No cell clumps should be visible after resuspension of the pellet.

2. **Add 10 ml Cell Lysis Solution and invert the tube gently 4-6 times to mix. Do not vortex, as this will result in shearing of genomic DNA.**
   If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

3. **Add 30 ml Neutralization Solution and invert the tube immediately but gently 4-6 times.**
   To avoid localized precipitation, immediately after addition of Neutralization solution mix the solution gently but thoroughly. The solution should become cloudy.

4. **Centrifuge the suspension in a centrifuge for 10 min at 2500 x g, RT.**
   A compact white pellet will form.
   During centrifugation, prepare the vacuum manifold and Maxi-columns:
   - Insert each Maxi-column into a luer connector on the luer adapter(s) in the vacuum manifold.

5. **Apply the supernatant from step 4 to the Maxi-column by decanting or pipetting.**

6. **Switch on vacuum source to draw the solution through the Maxi-columns, and then switch off vacuum source.**

7. **(Optional): Wash the Maxi-column by adding 20 ml Wash B Solution (not include). Switch on vacuum source. After the solution has moved through the Maxi-column, switch off vacuum source.**
   This step is necessary to remove trace nuclease activity when using endA+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α do not require this additional wash step.

8. **Wash the Maxi-column by adding 20 ml Wash A Solution. Switch on vacuum source to draw the wash solution through the Maxi-column, and then switch off vacuum source.**

9. **Transfer the Maxi-columns to a centrifuge tube. Centrifuge for 5 min at 2500 x g, RT.**
   IMPORTANT: This extra spin is necessary to remove residual Wash A Solution. Residual ethanol from Wash A Solution may inhibit subsequent enzymatic reactions.

10. **Place the Maxi-column in a clean 50 ml centrifuge tube.**
    To elute DNA, add 4 ml Elution Buffer (10 mM Tris·Cl, pH 8.5) or Water to the center of the Maxi-column, let stand for 1 min, and centrifuge for 5 min at 2500 x g.

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 Plasmid Purification spin column, modify the wash step by incubating the column for 5 minutes at room temperature after adding 750 ul Wash A (Midi:3 ml, Maxi:20 ml) Solution and then centrifuging. Ensure that two wash steps are carried out prior to elution.

Comments and suggestions	
b) Nuclease contamination	When using *endA*+ host strains such as HB101 and its derivatives, the JM series, or any wild-type strain, ensure that the wash step with Wash B Solution is performed.
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.

Special Applications

Purification of low-copy plasmids and cosmids

All Nucleogen Plasmid Purification protocols in this handbook can be used for preparation of low-copy-number plasmid or cosmids from overnight E. coli cultures grown in LB medium.

Only two slight modifications to the protocols are required:

- The wash step with Wash B Solution is required for all strains.
- When plasmid or cosmids are >10 kb, pre-heat Elution Buffer or water to 70°C prior to eluting DNA from the Nucleogen Plasmid Purification membrane.

Note: When using 10 ml (Midi; 100 ml, Maxi; 500 ml) culture volume, it is recommended to double the volumes of Cell Resuspension Solution, Cell Lysis Solution, and Neutralization Solution used.

Purification of very large plasmids (>50 kb)

Plasmids >50 kb elute less efficiently from silica than smaller plasmids,

Additional Protocol

Protocol for plasmid DNA from Yeast using the Mini Kit

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

1. **Inoculate a single colony into 2–5 ml of the appropriate selective media and grow the culture for 16–24 h at 30°C.**

2. **Harvest the cells by centrifugation for 5 min at 5000 x g and resuspend cells in 250 ul Cell Resuspension Solution containing 0.1 mg/ml RNase A. Transfer the resuspended cell to a microcentrifuge tube.**

3. **Add 50–100 ul of acid-washed glass beads (Sigma G-8772, not include) and vortex for 5 min. Let stand to allow the beads to settle. Transfer supernatant to a fresh microcentrifuge tube.**

4. **Add 250 ul Cell Lysis Solution to the tube and invert gently 4–6 times to mix. Incubate at room temperature for 5 min.**

5. **Add 350 ul Neutralization Solution to the tube and invert immediately but gently 4–6 times.**

6. **Centrifuge the lysate for 10 min at maximum speed in a tabletop microcentrifuge (13,000 rpm or ≥10,000 x g). Meanwhile, place a Spin Column in a 2 ml collection tube.**

7. **Transfer the cleared lysate from step 6 to Nucleogen Plasmid Purification Spin Column.**

8. **Centrifuge for 30–60 s (13,000 rpm or ≥10,000 x g). Discard flow-through.**

9. **Wash Spin Column by adding 0.75 ml of Wash A Solution and centrifuging 30–60 s (13,000 rpm or ≥10,000 x g).**

10. **Discard flow-through and centrifuge for an additional 1 min to remove residual Wash A Solution (13,000 rpm or ≥10,000 x g).**
    IMPORTANT: Residual Wash A Solution will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Wash A Solution may inhibit subsequent enzymatic reactions.

11. **Place Nucleogen Plasmid Purification Spin Column in a clean microcentrifuge tube. To elute DNA, add 25 ul of Elution Buffer (10 mM Tris·Cl, pH 8.5) or water to the center of each Spin Column, let stand for 1 min, and centrifuge for 1 min.**
    Typical yield is up to 1 ug. For subsequent PCR, use 0.1-1.0 ul of the eluate. For subsequent transformation into E. coli, 2–3 ul of eluate yields about 30 colonies.

Protocol for Plasmid DNA from *Bacillus subtilis* using the Mini Kit

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

1. **Pick a single colony from a selective plate and inoculate a culture of 10 ml LB medium containing the appropriate antibiotic. Grow the culture at 37°C with vigorous shaking (~240 rpm) for approximately 4–6 h until O.D.600 = 0.8–1.2.**
   A short growth time is recommended for two reasons: 1. a lower cell density overcomes incomplete lysis due to the thick peptidoglycan cell wall of Gram-positive bacteria, and 2. *Bacillus* spp. secrete large amounts of nucleases into the media and produce lysis-resistant spores during post-exponential growth.

2. **Harvest the cells by centrifugation at 3000 x g for 15 min at 4°C.**

3. **Resuspend pelleted bacterial cells in 250 ul Cell Resuspension Solution containing lysozyme at a final concentration of 1 mg/ml.**
   Ensure that RNase A has been added to Cell Resuspension Solution. No cell clumps should be visible after resuspension of the pellet.

4. **Incubate at 37°C for 10 min.**

5. **Add 250 ul Cell Lysis Solution and gently invert the tube 4–6 times to mix.**
   Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

6. **Add 350 ul Neutralization Solution and invert the tube immediately but gently 4–6 times.**
   To avoid localized precipitation, mix the solution gently but thoroughly, immediately after addition of Neutralization Solution. The solution should become cloudy.

7. **Centrifuge at ≥10,000 x g for 10 min.** A compact white pellet will form.

8. **Apply the supernatant from step 7 to the Nucleogen Plasmid Purification Spin Column.**

9. **Centrifuge at ≥10,000 x g for 30–60 s. Discard the flow-through.**

10. **Wash the Spin Column by adding 0.5 ml Wash B Solution and centrifuge at ≥10,000 x g for 30–60 s. Discard the flow-through.**
    This step removes trace nuclease activity, and is necessary for all *B. subtilis* strains.

11. **Wash the Spin Column by adding 0.75 ml Wash A Solution and centrifuge at ≥10,000 x g for 30–60 s.**

12. **Discard the flow-through, and centrifuge at ≥10,000 x g for an additional 1 min to remove residual Wash A Solution.**
    IMPORTANT: Residual Wash A Solution will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Wash A Solution may inhibit subsequent enzymatic reactions.

13. **Place the Nucleogen Plasmid Purification Spin Column in a clean microcentrifuge tube. To elute DNA, add 50 ul Elution Buffer (10 mM Tris·Cl, pH 8.5) or water to the center of each Spin Column, let stand for 1 min, and centrifuge for 1 min.**

Protocol for plasmid DNA from *Agrobacterium* using the Mini Kit

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

1. **Grow a*grobacteria* containing the vector on YEB plates for 2 days at 28°C.**

2. **Inoculate a single colony into 10 ml liquid YEB medium and grow the culture overnight at 28°C with 200 rpm shaking to an O.D.600 = 1.2–1.5.**

3. **Harvest the cells from a 10 ml aliquot by centrifugation for 15 min at 3500 rpm or 1500 x g, and resuspend in 250 ul Cell Resuspension Solution containing 0.1 mg/ml RNase A.** Depending on the host strain, doubling the volumes of Cell Resuspension Solution, Cell Lysis Solution, and Neutralization Solution, or increasing the culture volume to 15 ml, may sometimes enhance plasmid yield.

4. **Add 250 ul Cell Lysis Solution to the tube and invert gently 4–6 times to mix.**

5. **Add 350 ul Neutralization Solution to the tube and invert immediately but gently 4–6 times.**

6. **Centrifuge the lysate for 10 min at 13,000 rpm or ≥10,000 x g in a tabletop microcentrifuge.**

7. **Transfer the cleared lysates from step 6 to the Nucleogen Plasmid Purification Spin Column.**

8. **Centrifuge 30–60 s (13,000 rpm or ≥10,000 x g). Discard flow-through.**

9. **Wash the Spin Column by adding 0.5 ml of Wash B Solution and centrifuging 30–60 s (13,000 rpm or ≥10,000 x g). Discard flow-through.**

10. **Wash the Spin Column by adding 0.75 ml of Wash A Solution and centrifuging 30–60 s (13,000 rpm or ≥10,000 x g).**

11. **Discard flow-through and centrifuge for an additional 1 min at 13,000 rpm or ≥10,000 x g to remove residual Wash A Solution.**
    IMPORTANT: Residual Wash A Solution will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Wash A Solution may inhibit subsequent enzymatic reactions.

13. **Place the Nucleogen Plasmid Purification Spin Column in a clean microcentrifuge tube. To elute DNA, add 50 ul of Elution Buffer (10 mM Tris·Cl, pH 8.5) or water to the center of each Spin Column, let stand for 1 min, and centrifuge for 1 min.**

Medium composition YEB medium (1 liter): To prepare 1 liter YEB medium: In 600 ml water, dissolve 5 g beef extract, 1 g yeast extract, 5 g peptone, 5 g sucrose, and pH to 7.2. For YEB plates, add 18 g bactoagar. Bring volume up to 1 liter with water and autoclave. Add sterile solutions of kanamycin, rifampicin, and MgSO4 to final concentrations of 100 mg/liter, 50 mg/liter, and 2 mM, respectively.

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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