GeneJET[™] Plasmid Midiprep Kit user guide

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Revision history: MAN0012653 B (English)

Revision	Date	Description
В	29 May 2025	 Updated to the current document template, with associated updates to the limited license information, warranty, trademarks, and logos. The contents and storage table was updated to include additional instructions for storage of purification columns.
A.0	17 October 2015	New document for the GeneJET [™] Plasmid Midiprep Kit.

The information in this guide is subject to change without notice.

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Product information

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. SDSs are available from thermofisher.com/support.

Product description

The GeneJET[™] Plasmid Midiprep Kit is designed for large-scale isolation of high quality plasmid DNA from recombinant E.coli cultures. The kit utilizes silica-based membrane technology in the form of convenient spin columns. Each prep recovers up to 200 µg of high copy number plasmid DNA. The purified plasmid DNA can be used in a wide variety of molecular biology procedures such as restriction endonuclease digestion, PCR, cloning, transformation, automated sequencing, *in vitro* transcription, and transfection of robust cell lines.

Technology overview

Pelleted bacterial cells are resuspended and subjected to SDS/alkaline lysis to liberate plasmid DNA. The resulting lysate is neutralized allowing denatured plasmid DNA to reanneal while cell debris such as proteins, chromosomal DNA and SDS precipitate out of solution. The resulting precipitate is pelleted by centrifugation and the supernatant containing plasmid DNA is loaded onto the purification column. The high salt concentration of the lysate creates the appropriate conditions for plasmid DNA binding to the silica membrane in the spin column. The adsorbed DNA is washed to remove contaminants and eluted with the Elution Buffer.

Contents and storage

Component	Cat. No. K0481 (25 reactions)	Cat. No. K0482 (100 reactions)	Storage
Resuspension Solution ^[1]	60 mL	240 mL	
Lysis Solution	60 mL	240 mL	15–25°C
Neutralization Solution	60 mL	240 mL	
Endotoxin Binding Reagent	15 mL	60 mL	2–8°C
Wash Solution I (concentrated)	90 mL	360 mL	15–25°C

Table 1 Components of the GeneJET[™] Plasmid Midiprep Kit

Component	Cat. No. K0481 (25 reactions)	Cat. No. K0482 (100 reactions)	Storage
Wash Solution II (concentrated)	90 mL	2 × 180 mL	15–25°C
RNase A Solution	2 × 1.2 mL	8 × 1.2 mL	15–25°C (unopened vial) -20°C (opened vial)
Elution Buffer (10 mM Tris- HCl, pH 8.5)	18 mL	60 mL	15–25°C
GeneJET [™] Midi Purification Columns (preassembled with collection tubes)	25	100	15–25°C For better long-term performance store at 2°C to 8°C.
Collection tubes (15 mL)	25	100	15–25°C

Table 1	Components of the GeneJET Plasmid Midiprep Kit	(continued	J)
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^[1] Following the addition of RNase A Solution, the Resuspension Solution should be stored at 2–8°C and is stable for 6 months.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Adjustable micropipettor	MLS
Thermo heating-blocks or water bath (adjustable to 56°C)	MLS
Vortex	MLS
Microcentrifuge of \ge 48,000 × <i>g</i> with rotor for appropriate centrifuge tubes, or centrifuge with swinging bucket rotor capable of 4,000–5,000 × <i>g</i>	MLS
Vacuum manifold	MLS
RNase-free pipette tips (sterile)	MLS
Disposable gloves	MLS
Ethanol, 96–100% (molecular biology grade)	MLS
Isopropanol	MLS
(Optional) T7 DNA Polymerase	EP0081
(Optional) GeneJET™ PCR Purification Kit	K0701

Procedural guidelines

IMPORTANT! Tightly seal the bag containing purification columns after each use.

- Wear gloves when handling the Lysis Solution and Wash Solution I. These solutions contain irritants and are harmful if contacted with skin, inhaled, or swallowed.
- Harvest the bacterial culture by centrifugation at +4°C. All others centrifugation steps should be carried out at room temperature.

Recommendations for bacterial strains

High-quality plasmid DNA can be obtained from various E.coli strains including DH10B, DH5 α , XL1-Blue, JM109, JM107, and TOP10.

Recommendations for culture media

The protocol outlined in this manual is optimized for use with bacterial cultures grown in Luria-Bertani (LB) medium to a cell density of approximately 2-3 (OD₆₀₀ = 2-3).

IMPORTANT! The use of rich growth media is not recommended.

Recommendations for culture volume

Generally, 50 mL of overnight bacterial culture grown in LB medium is sufficient for good yield of highcopy and low-copy number plasmid DNA. However, it is important not to exceed the recommended cell mass (culture volume \times OD₆₀₀), because it may decrease the quality of isolated DNA.

The maximum culture volume to use can be determined using the formula below:

Maximum culture volume (mL) = $150/OD_{600}$

Table 2	Copy	numbers	of various	vectors
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High-copy	Low-copy	Very low-copy	
300–700 copies per cell	10–50 copies per cell	Up to 5 copies per cell	
 pUC vectors pBluescript vectors pGEM vectors pTZ vectors pJET vectors 	pBR322 and derivativespACYC and derivatives	pSC101 and derivatives	



Before first use of the kit

1. Add the indicated volume of isopropanol to Wash Solution I (concentrated) and ethanol (96–100%) to Wash Solution II before first use.

Component	25 reactions		100 reactions	
Component	Wash Solution I	Wash Solution II	Wash Solution I	Wash Solution II
Concentrated wash solution	90 mL	90 mL	360 mL	2 × 180 mL
Isopropanol	30 mL	_	120 mL	_
Ethanol (96–100%)	_	150 mL	_	2 × 300 mL
Total volume	120 mL	240 mL	480 mL	2 × 480 mL

2. Mark the checkbox on the bottlecap to indicate that isopropanol or ethanol has been added to the bottle.

Before each use of the kit

- 1. Check Lysis Solution and the Neutralization Solution for salt precipitation before each use.
- 2. Redissolve any precipitate by warming the solution at 37°C, then cool back down to 25°C before use.





This manual provides optimized protocols for plasmid DNA purification using low speed and high speed centrifuges, as well as using vacuum manifolds.

Table 3 Protocols depending on the equipment used

Equipment	Centrifugation protocol	Vacuum protocol
Low speed centrifuge (up to 5,000 x <i>g</i>)	"Purify plasmid DNA using low speed centrifugation" on page 11	"Purify DNA using vacuum manifold" on page 14
High speed centrifuge (up to 48,000 x <i>g</i>)	"Purify plasmid DNA using high speed centrifugation" on page 12	"Purify DNA using vacuum manifold" on page 14

Prepare resuspension solution

1. Add the RNase A Solution provided to the Resuspension Solution, then mix thoroughly.

Note: After the addition of RNase A, the Resuspension Solution is stable for 6 months when stored at 4° C.

 If the kit will be used infrequently, divide the Resuspension Solution into an appropriate number of aliquots and supplement one aliquot with 40 μL of RNase A per 1 mL of Resuspension Solution to create a working solution.

Note: Sequential working solutions can be prepared by supplementing an aliquot of the Resuspension Solution with RNase A.

Store the remaining RNase A at -20°C.



Grow bacterial culture

- 1. To prepare the bacterial culture, pick a single colony from a freshly streaked selective plate to inoculate 1–5 mL of LB medium supplemented with the appropriate antibiotic.
- 2. Incubate for approximately 8 hours at 37°C while shaking at 200–250 rpm.
- 3. Dilute the starter culture from 1:1,000 to 1:10,000 in LB medium.
- 4. Incubate for 12–16 hours (overnight) at 37°C while shaking at 200–250 rpm.

Note: The recommended bacterial culture optical density for plasmid DNA isolation is approximately 2-3 (OD₆₀₀ = 2-3).

5. Harvest the cells by centrifugation at $5,000 \times g$ for 10 minutes. Discard the supernatant.

Note: For best results calculate the maximum volume of cell culture to use. See page 7.

The bacterial pellet can be used immediately or stored at -20°C.

Lyse cells

1. Resuspend pelleted cells in 2 mL of Resuspension Solution by vortexing or pipetting up and down until no cell clumps remain.

Note: Ensure that the RNase A Solution has been added to the Resuspension Solution. See "Prepare resuspension solution" on page 9.

2. Add 2 mL of Lysis Solution, then mix gently by inverting the tube 4–6 times until the solution becomes viscous and slightly clear. Incubate for 3 minutes at room temperature.

IMPORTANT!

- · Do not vortex to avoid shearing chromosomal DNA.
- . Do not incubate for more than 3 minutes to avoid denaturation of supercoiled plasmid DNA.
- **3.** Proceed to "Purify plasmid DNA using low speed centrifugation" on page 11, "Purify plasmid DNA using high speed centrifugation" on page 12, or "Purify DNA using vacuum manifold" on page 14.

Purify plasmid DNA using low speed centrifugation

Clear lysate

- 1. To lysed cells, add 2 mL of Neutralization Solution, then mix immediately by inverting the tube 5–8 times.
- 2. Add 0.5 mL of the Endotoxin Binding Reagent, then mix immediately by inverting the tube 5– 8 times. Incubate 5 minutes at room temperature.

Note: After the addition of the Neutralization Solution and Endotoxin Binding Reagent it is important to mix the contents of the tube gently, but thoroughly, to avoid localized precipitation of bacterial cell debris. The neutralized bacterial lysate should appear cloudy and contain white precipitate.

- 3. Add 3 mL of ethanol (96%). Mix immediately by inverting the tube 5–6 times.
- 4. To pellet cell debris and chromosomal DNA, centrifuge at 4,000-5,000 \times g for 40 minutes.

Note: Use recommended centrifugation speed.

5. Transfer supernatant to a 15 mL tube by decanting or pipetting.

Note: Avoid disturbing or transferring the white precipitate.

- 6. Add 3 mL of ethanol (96%). Mix immediately by inverting the tube 5–6 times.
- 7. Proceed to page 11.

Bind DNA

1. Transfer part of the sample (approximately 5.5 mL) to the supplied pre-assembled column with collection tube (15 mL).

Note: Do not overfill the column.

2. Centrifuge at 2,000 \times *g* for 3 minutes in a swinging bucket rotor. Discard the flow-through, then place the column back into the same collection tube.

Note: Close the bag with columns tightly after use.

- **3.** To process any remaining lysate through the purification column, repeat step 1 and step 2.
- 4. Proceed to page 12.



Wash DNA

- 1. Add 4 mL of Wash Solution I supplemented with isopropanol (see "Before first use of the kit" on page 8) to the purification column.
- 2. Centrifuge at $3,000 \times g$ for 2 minutes in a swinging bucket rotor. Discard the flow-through, then place the column back into the same collection tube.
- **3.** Add 4 mL of Wash Solution II supplemented with ethanol (see "Before first use of the kit" on page 8) to the purification column.
- 4. Centrifuge at $3,000 \times g$ for 2 minutes in a swinging bucket rotor. Discard the flow-through, then place the column into the same collection tube.
- 5. Repeat step 3 and step 4.
- 6. Centrifuge at $3,000 \times g$ for 5 minutes in a swinging bucket rotor to remove residual wash solution. Discard the collection tube containing the flow-through.

Elute DNA

- 1. Transfer the column to a fresh collection tube (15 mL).
- 2. Add 0.35 mL of Elution Buffer to the center of the purification column membrane.
- **3.** To elute plasmid DNA, incubate for 2 minutes at room temperature, then centrifuge at $3,000 \times g$ for 5 minutes. Discard the purification column.

Note: To increase the concentration of eluted DNA, the volume of the Elution Buffer can be reduced to 0.25 mL. Be aware that lower volumes of Elution Buffer will decrease the overall yield of eluted DNA. To increase the overall DNA yield by 20–30% an additional elution step (optional) with Elution Buffer (0.15 mL) may be used.

Use the purified plasmid DNA in downstream applications or store at -20°C.

Purify plasmid DNA using high speed centrifugation

Clear lysate

- 1. To lysed cells, add 2 mL of Neutralization Solution, then mix immediately by inverting the tube 5–8 times.
- 2. Add 0.5 mL of the Endotoxin Binding Reagent, then mix immediately by inverting the tube 5– 8 times. Incubate 5 minutes at room temperature.

Note: After the addition of the Neutralization Solution and Endotoxin Binding Reagent it is important to mix the contents of the tube gently, but thoroughly, to avoid localized precipitation

of bacterial cell debris. The neutralized bacterial lysate should appear cloudy and contain white precipitate.

- 3. Add 3 mL of ethanol (96%). Mix immediately by inverting the tube 5–6 times.
- 4. To pellet cell debris and chromosomal DNA, centrifuge at 4,000-5,000 \times g for 40 minutes.

Note: Use recommended centrifugation speed.

5. Transfer supernatant to a 15 mL tube by decanting or pipetting.

Note: Avoid disturbing or transferring the white precipitate.

- 6. Add 3 mL of ethanol (96%). Mix immediately by inverting the tube 5–6 times.
- 7. Proceed to "Bind DNA" on page 13.

Bind DNA

1. Transfer part of the sample (approximately 5.5 mL) to the supplied column pre-assembled with a collection tube (15 mL).

Note: Do not overfill the column.

2. Centrifuge at 2,000 \times *g* for 3 minutes in a swinging bucket rotor. Discard the flow-through, then place the column back into the same collection tube.

Note: Close the bag with columns tightly after use

- 3. For any remaining lysate, repeat step 2.
- 4. Proceed to "Wash DNA" on page 13.

Wash DNA

- 1. Add 4 mL of Wash Solution I supplemented with isopropanol (see "Before first use of the kit" on page 8) to the purification column.
- 2. Centrifuge at $3,000 \times g$ for 2 minutes in a swinging bucket rotor. Discard the flow-through, then place the column back into the same collection tube.
- **3.** Add 4 mL of Wash Solution II supplemented with ethanol (see "Before first use of the kit" on page 8) to the purification column.
- 4. Centrifuge at $3,000 \times g$ for 2 minutes in a swinging bucket rotor. Discard the flow-through, then place the column into the same collection tube.
- 5. Repeat step 3 and step 4.
- 6. Centrifuge at $3,000 \times g$ for 5 minutes in a swinging bucket rotor to remove residual wash solution. Discard the collection tube containing the flow-through.



Elute DNA

- 1. Transfer the column to a fresh collection tube (15 mL).
- 2. Add 0.35 mL of Elution Buffer to the center of the purification column membrane.
- **3.** To elute plasmid DNA, incubate for 2 minutes at room temperature, then centrifuge at $3,000 \times g$ for 5 minutes. Discard the purification column.

Note: To increase the concentration of eluted DNA, the volume of the Elution Buffer can be reduced to 0.25 mL. Be aware that lower volumes of Elution Buffer will decrease the overall yield of eluted DNA. To increase the overall DNA yield by 20–30% an additional elution step (optional) with Elution Buffer (0.15 mL) may be used.

Use the purified plasmid DNA in downstream applications or store DNA at -20°C.

Purify DNA using vacuum manifold

Clear lysate

To lysed cells, perform lysate clearing following "Clear lysate" on page 11 of "Purify plasmid DNA using low speed centrifugation" on page 11 or "Clear lysate" on page 12 of "Purify plasmid DNA using high speed centrifugation" on page 12.

Bind DNA

- 1. Prepare the vacuum manifold according to the supplier's instructions.
- 2. Place the purification column(s) onto the manifold.
- 3. Transfer part of the sample (approximately 5.5 mL) to the purification column.

Note: Be careful not to overfill the column.

4. Apply vacuum to draw the solution through the column. Switch off the vacuum after the solution has passed through the column.

Note: Close the bag with columns tightly after use.

5. To process the remaining lysate through the purification column, repeat step 4.

Wash DNA

- 1. Add 4 mL of Wash Solution I supplemented with isopropanol (see "Before each use of the kit" on page 8) to the purification column.
- 2. Apply the vacuum to draw the solution through the column. Switch off the vacuum after the solution has passed through the column.
- **3.** Add 4 mL of Wash Solution II supplemented with ethanol (see "Before each use of the kit" on page 8) to the purification column.
- 4. Apply the vacuum to draw the solution through the column. Switch off the vacuum after the solution has passed through the column.
- 5. Repeat step 3 and step 4 once.
- 6. To dry the column, transfer the purification column into a fresh 15 mL collection tube (provided). Centrifuge at $3,000 \times g$ for 5 minutes in a swinging bucket rotor.

Elute DNA

- 1. Transfer the column to a fresh 15 mL collection tube.
- 2. Add 0.35 mL of Elution Buffer to the center of the column membrane.
- **3.** Incubate the column for 2 minutes at room temperature, then centrifuge at $3,000 \times g$ for 5 minutes in a swinging bucket rotor to elute plasmid DNA. Discard the purification column.

Note: If more concentrated DNA samples are required, the volume of Elution Buffer added to the column can be reduced to 0.25 mL. Be aware that smaller volumes of Elution Buffer will decrease the overall yield of eluted DNA. To increase the overall DNA yield by 20–30%, an additional elution step (optional) with Elution Buffer (0.15 mL) may be used.

Use the purified plasmid DNA for downstream applications or store the DNA at -20°C.



Troubleshooting

Observation	Possible cause	Recommended action
Low plasmid DNA yield	Incomplete bacterial cell lysis.	It is essential that the cell pellet is completely resuspended in the Resuspension Solution prior to lysis. Cell clumps should not be visible before the addition of Lysis Solution.
		Check the Lysis Solution for salt precipitation before each use. Redissolve any precipitate by warming the solution to 37°C, then mix well and cool to 25°C before use.
		Do not use more biomass than recommended. See page 7.
	Isopropanol was not added to Wash Solution I.	Ensure that isopropanol was added to Wash Solution I before the first use. See page 8.
	Ethanol was not added to Wash Solution II.	Ensure that ethanol was added to Wash Solution II before the first use. See page 8.
	Column clogs during the filtration procedure.	Reduce the volume of cell culture biomass processed per column.
		Avoid transferring pelleted cell debris to the purification column when loading the lysate to the column.
	Suboptimal cell culture volume.	Calculate the maximum culture volume using the following formula: Maximum culture volume (mL) = $150/OD_{600}$.
	Old bacterial culture.	Prepare a new starter culture by inoculating a freshly isolated single bacterial colony in antibiotic-containing growth medium and grow bacteria. See page 10.
	Inappropriate centrifuge speed.	Make sure that the recommended centrifuge speed was used during purification. It is very important to meticulously adhere to the centrifugation protocol.
Suboptimal A ₂₆₀ /A ₂₈₀ ratio	Purification of plasmid DNA is not efficient.	Reduce the volume of cell culture. See page 7.
Genomic DNA contamination	Samples were vigorously vortexed or shaken during cell lysis or neutralization steps.	To avoid genomic DNA contamination, mix the solution (see step 2 of page 10, step 1 of page 11, or step 1 of page 12) by gently inverting the tubes 4–8 times.
		Do not allow the cell lysis step (see step 2 of page 10) to proceed for more than 3 minutes.
		Do not cultivate cells longer than 16 hours in LB media.
		Residual genomic DNA can be removed from purified plasmid DNA using T7 DNA Polymerase (Cat. No. EP0081).



Observation	Possible cause	Recommended action
RNA contamination	RNase A Solution was not added to the Resuspension Solution before the first use.	Ensure that the RNase A Solution was added to the Resuspension Solution. See page 9.
Purified prep contains additional plasmid forms	Plasmid DNA denatured during cell lysis.	Denatured plasmid DNA migrates ahead of supercoiled DNA and is not suitable for enzymatic manipulations such as restriction digestion. To avoid denaturation, do not lyse the cells (see step 2 of page 10) for more than 3 minutes.
Inhibition of downstream enzymatic reactions	Purified plasmid DNA contains ethanol.	Dry the purification column sufficiently prior to the DNA elution (step 6 of page 12, step 5 of page 13, or step 5 of page 15).
No or low yields of in vitro transcription reaction products	Purified plasmid DNA contains RNase A.	If the purified DNA is used for in vitro transcription, linearized plasmid DNA can be repurified using the GeneJET [™] PCR Purification Kit (K0701) or by phenol/chloroform extraction.







WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit thermofisher.com/support.



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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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