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GeneJET™ Plasmid Maxiprep Kit

USER GUIDE

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Revision	Date	Description
B	15 June 2025	• Updated to the current record template, with associated updates to the limited license information, warranty, trademarks, and logos. • The contents and storage table was updated to include added instructions for storage of purification columns.
A.0	17 October 2015	Baseline.

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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 Maxiprep 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 750 µg of high copy plasmid DNA that 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 to re-anneal plasmid DNA and precipitate proteins and chromosomal DNA. Cell debris and SDS precipitate are pelleted by centrifugation. The supernatant containing plasmid DNA is loaded onto the purification column. The high salt concentration of the lysate creates 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

Table 1 Components of the GeneJET™ Plasmid Maxiprep Kit

Component	Cat. No. K0491 (10 reactions)	Cat. No. K0492 (25 reactions)	Storage
Resuspension Solution[1]	72 mL	180 mL	15–25°C
Lysis Solution	72 mL	180 mL	
Neutralization Solution	72 mL	180 mL	
Endotoxin Binding Reagent	9.6 mL	24 mL	2–8°C
Wash Solution I (concentrated)	75 mL	180 mL	15–25°C

Table 1 Components of the GeneJET Plasmid Maxiprep Kit *(continued)*

Component	Cat. No. K0491 (10 reactions)	Cat. No. K0492 (25 reactions)	Storage
Wash Solution II (concentrated)	75 mL	180 mL	15–25°C
RNase A Solution	3 × 1.0 mL	6 × 1.2 mL	15–25°C (unopened vial) -20°C (opened vial)
Elution Buffer (10 mM Tris-HCl, pH 8.5)	18 mL	45 mL	15–25°C
GeneJET™ Maxi Purification Columns preassembled with collection tubes (50 mL)	10	25	15–25°C For better long-term performance store at 2°C to 8°C.
Collection tubes (50 mL)	10	25	15–25°C

[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**
Centrifuge of ≥48,000 × *g* with rotor for appropriate centrifuge tubes, or centrifuge with swinging bucket rotor capable of 2,000–5,000 × *g*	**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, DH5α, 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 (OD_{600} = 2–3).

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

Recommendations for cell culture volume

Generally, 250 mL of overnight bacterial culture grown in LB medium is sufficient for good yield of high-copy and low-copy number plasmid DNA. However, it is important not to exceed the recommended cell mass (culture volume × OD_{600}), 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) = 750/OD_{600}

Table 2 Copy numbers of various vectors

High-copy 300–700 copies per cell	Low-copy 10–50 copies per cell	Very low-copy Up to 5 copies per cell
• pUC vectors • pBluescript vectors • pGEM vectors • pTZ vectors • pJET vectors	• pBR322 and derivatives • pACYC 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	10 reactions		25 reactions	
	Wash Solution I	Wash Solution II	Wash Solution I	Wash Solution II
Concentrated wash solution	75 mL	75 mL	180 mL	180 mL
Isopropanol	25 mL	–	60 mL	–
Ethanol (96–100%)	–	125 mL	–	300 mL
Total volume	**100 mL**	**200 mL**	**240 mL**	**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.

Methods

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.

2. 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 (OD_{600} = 2–3).

5. Proceed to “Lyse cells” on page 10.

Lyse cells

1. Harvest the cells by centrifugation at 5,000 × *g* for 10 minutes. Discard the supernatant.

   Note:
   - For best results calculate the maximum volume of cell culture to use. See "Recommendations for cell culture volume" on page 7.
   - The bacterial pellet can be used immediately or stored at -20°C.

2. Resuspend pelleted cells in 6 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.

3. Add 6 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.

4. Proceed to "Purify plasmid DNA using low speed centrifugation" on page 11, "Purify plasmid DNA using high speed centrifugation" on page 13, or "Purify plasmid DNA using vacuum manifold" on page 15.

Purify plasmid DNA using low speed centrifugation

Clear lysate

1. To lysed cells (see “Lyse cells” on page 10), add 6 mL of Neutralization Solution, then mix immediately by inverting the tube 5–8 times.
2. Add 0.8 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 6 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 × *g* for 40 minutes.

   Note: Use recommended centrifugation speed.

5. Transfer supernatant to a 50 mL tube by decanting or pipetting.

   Note: Avoid disturbing or transferring the white precipitate.

6. Add 6 mL of ethanol (96%). Mix immediately by inverting the tube 5–6 times.
7. Proceed to “Bind DNA” on page 11.

Bind DNA

1. Transfer part of the sample (approximately 20 mL) to the purification column.

   Note: Do not overfill the column.

2. Centrifuge at 2,000 × *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 “Wash DNA” on page 12.

Wash DNA

1. Add 8 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 × *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 8 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 × *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 × *g* for 5 minutes in a swinging bucket rotor to remove residual wash solution. Discard the collection tube containing the flow-through.

7. Proceed to “Elute DNA” on page 12.

Elute DNA

1. Transfer the column to a fresh collection tube (50 mL).

2. Add 1 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 × *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 0.7 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 (0.5 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 (see “Lyse cells” on page 10), add 6 mL of Neutralization Solution, then mix immediately by inverting the tube 5–8 times.

2. Add 0.8 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. To pellet cell debris and chromosomal DNA, centrifuge at 48,000 × *g* for 20 minutes.

   Note: Use recommended centrifugation speed.

4. Transfer supernatant to a 50 mL tube by decanting or pipetting.

   Note: Avoid disturbing or transferring the white precipitate.

5. An equal volume of ethanol (96%) must be added to the centrifuged supernatant (ratio must be 1:1). Mix immediately by inverting the tube 5–6 times.

6. Proceed to “Bind DNA” on page 13.

Bind DNA

1. Transfer part of the sample (approximately 20 mL) to the purification column.

   Note: Do not overfill the column.

2. Centrifuge at 2,000 × *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 1 and step 2.

4. Proceed to “Wash DNA” on page 14.

Wash DNA

1. Add 8 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 × *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 8 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 × *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 × *g* for 5 minutes in a swinging bucket rotor to remove residual wash solution. Discard the collection tube containing the flow-through.
7. Proceed to "Elute DNA" on page 14.

Elute DNA

1. Transfer the column to a fresh collection tube (50 mL).
2. Add 1 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 × *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 (0.5 mL) may be used.

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

Purify plasmid DNA using vacuum manifold

Clear lysate

To lysed cells (see “Lyse cells” on page 10), perform lysate clearing following “Clear lysate” on page 11 of page 11 or “Clear lysate” on page 13 of page 13.

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 20 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.
6. Proceed to “Wash DNA” on page 15.

Wash DNA

1. Add 8 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 8 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, apply the vacuum for 10 minutes or transfer the purification column into a fresh 15 mL collection tube (provided). Centrifuge at 3,000 × *g* for 5 minutes in a swinging bucket rotor.
7. Proceed to “Elute DNA” on page 16.

Elute DNA

1. Transfer the column to a fresh 50 mL collection tube.
2. Add 1 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 × *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 0.7 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 (0.5 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 "Recommendations for cell culture volume" on page 7.
	Isopropanol was not added to Wash Solution I.	Ensure that isopropanol was added to Wash Solution I before the first use. See "Before first use of the kit" on page 8.
	Ethanol was not added to Wash Solution II.	Ensure that ethanol was added to Wash Solution II before the first use. See "Before first use of the kit" on 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) = $750/OD_{600}$.
	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 "Grow bacterial culture" on page 9.
	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 "Recommendations for cell culture volume" on 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 3 of "Lyse cells" on page 10, step 1 of "Clear lysate" on page 11, or step 1 of "Clear lysate" on page 13) by gently inverting the tubes 4–8 times.
		Do not allow the cell lysis step (see step 3 of "Lyse cells" on 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 “Prepare resuspension solution” on 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 3 of “Lyse cells” on 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 “Wash DNA” on page 12, step 5 of “Wash DNA” on page 14, or step 5 of “Wash DNA” on 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.

Safety

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**.

Documentation and support

Customer and technical support

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

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