Protech Technology

GeneBond™
MidiPrep/ MaxiPrep
Plasmid Purification Kit

Innovative Tools for Nucleic Acid Purification

This kit is for Research Use Only (RUO).
Not for diagnostic or any other human in vivo procedures

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Kit Contents

Component	Midiprep		Maxiprep
Cat No.	MP660-10	MP660-25	MP680-10
Quantity	10 preps	25 preps	10 preps
Cell Resuspension Solution (Buffer L1)	40 mL	100 mL	100 mL
Cell Lysis Solution (Buffer L2)	40 mL	100 mL	100 mL
Neutralization Solution (Buffer L3)	40 mL	100 mL	100 mL
Equilibration Buffer (Buffer EQ)	100 mL	250 mL	300 mL
Wash Buffer (Buffer WB)	2×100 mL	500 mL	2×300 mL
Elution Buffer (Buffer EB)	50 mL	125 mL	150 mL
TE Buffer (TE)	15 mL	15 mL	30 mL
Columns	10 each	25 each	10 each

Storage

All components of the GeneBond™ MidiPrep/ MaxiPrep Plasmid Purification Kits are shipped at room temperature. Store all components at room temperature. Please store Cell Resuspension Solution (Buffer L1) at 4°C for longer storage.

Product Description

The GeneBond™ MidiPrep/ MaxiPrep Plasmid Purification Kits purify plasmid DNA by using a anion-exchange resin. The resin provides outstanding capacity with high resolution, high yield.

The negatively charged phosphates on the DNA backbone interact with the positive charges on the resin surface. The binding process is influenced by the temperature, salt concentration, and pH of the solutions. Under moderate salt conditions, the plasmid DNA remains bound to the resin, while impurities such as RNA, proteins, and carbohydrates are washed away. The plasmid DNA is then eluted using a buffer with high salt conditions. You can complete the entire protocol in 3 hours.

Specifications

Specifications *	MidiPrep	MaxiPrep
Starting culture volume	15–25 mL	100–200 mL
Elution Volume	5 mL	15 mL
Expected DNA Yield***	>100 µg	>500 µg
Expected DNA Quality ($OD_{260/280}$)	~1.9	~1.9

* Specifications and results are based on high copy number plasmids.

** Binding capacity depends on plasmid copy number,

type and size, and volume of bacterial culture used.

*** DNA yield depends on plasmid copy number, type and size; bacterial strain; and growth conditions.

Downstream Applications

The purified DNA is suitable for downstream applications requiring the highest purity, such as:

- Transfection of mammalian cells
- Automated and manual DNA sequencing
- PCR amplification
- *In vitro* transcription
- Bacterial cell transformation
- Cloning
- Labeling

BEFORE STARTING

The section is instructions for cultivating the overnight cell culture and determining the appropriate quantities of starting bacterial cultures based on the plasmid copy number in use. ***Note:** *Some buffers in the GeneBond™ MidiPrep/ MaxiPrep Plasmid Purification Kits contain hazardous chemicals. Always wear a laboratory coat, disposable gloves, and eye protection when handling the buffers.*

BACTERIAL CULTURE

Harvest transformed *E. coli* cells overnight in LB medium with the appropriate antibiotic. The culture should reach a cell density of approximately 10^9 cells/mL, where OD600 is around 2.0.

PLASMID TYPE AND COPY NUMBER

GeneBond™ MidiPrep/ MaxiPrep Plasmid Purification Kits is apply for purifying high copy number plasmids and provide the best results with a typical yield of 2-6 μg DNA/ mL from an overnight culture. Yields from low copy number plasmids are dependent upon culture conditions and vector/ host strain combinations. Please follow the direction use a higher volume of cell culture for isolating low copy number plasmids. Recommended volumes of cell culture for plasmid

DNA purification are listed below.

Plasmid Copy Number	MidiPrep	MaxiPrep
High-copy number plasmid	15-25 mL	100-200 mL
Low-copy number plasmid	25-100 mL	250-500 mL

*For MaxiPreps of low copy number plasmids from bacterial cultures >200 mL, use twice the volumes specified in the protocol for Cell Resuspension Solution (Buffer L1), Cell Lysis Solution (Buffer L2), and Neutralization Solution (Buffer L3). ***Note:** *If the provided buffers are insufficient for the isolation of low copy number plasmids, you may purchase additional buffers*.

Recommendations for obtain the best results:

- Maintain a sterile workspace and equipment to avoid DNase contamination.
- Ensure that no DNase is introduced into the sterile solutions supplied with the kit.
- Perform all recommended wash steps.
- Use the TE Buffer (TE) provided or 10 mM Tris-HCl, pH 8.5 to resuspend the DNA pellet.

Buffer Preparation

Cell Lysis Solution (Buffer L2): If precipitates are present in Cell Lysis Solution (Buffer L2), briefly warm the solution at 37°C to dissolve them.

GeneBond™ MidiPrep Flow Chart

Equilibrate column

10 mL Equilibration Buffer
(Buffer EQ)

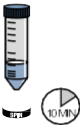
Prepare Cell Lysate

Harvest Cells

4, 000 x *g* , 10 min

4 mL Cell Resuspension
Solution (Buffer L1)

4 mL Cell Lysis Solution
(Buffer L2)



4 mL Neutralization Solution
(Buffer L3)

>12,000 x *g* , 10 min

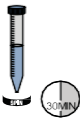
Bind and wash DNA

Load Column with lysate

10 mL Wash Buffer
(Buffer WB) x2

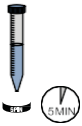
Elute and precipitate DNA

5 mL Elution Buffer
(Buffer EB)



3.5 mL Isopropanol

>15,000 x *g* , 30 min



3 mL 70% Ethanol

>15,000 x *g* , 5 min

200 µL TE Buffer

General Protocol for MidiPrep

The GeneBond™ MidiPrep Plasmid Purification Kits allows you to purify 100 µg of plasmid DNA from 15–25 mL overnight *E. coli* cultures in ~3 hours when cloning high copy number plasmids.

Materials Needed

- Overnight culture of transformed *E.coli* cells (page 5)
- Isopropanol
- 70% ethanol
- Sterile, microcentrifuge tubes
- Tubes or centrifuge bottles for harvesting cells
- 15 mL centrifuge tubes (elution tubes)
- Centrifuge with temperature control at 4°C

Equilibrating the Column

Add **10 mL Equilibration Buffer (Buffer EQ)** to the column. Allow the solution in the column to drain through gravity flow, proceed to the preparation of cell lysate while the column is in the equilibration process.

Preparing Cell Lysate

1. For **high copy number plasmids**, use 15–25 mL of an overnight LB culture per sample in a 50 mL tube.

***Note**: If you are using >25 mL of culture volume of high copy plasmids, add twice the amount of Cell Resuspension Solution (Buffer L1), Cell Lysis Solution (Buffer L2), and Neutralization Solution (Buffer L3) as directed in steps 3, 4, and 5, below, for best results*

For **low copy number plasmids**, use 25–100 mL of an overnight LB culture per sample in a 50 mL tube.

2. Harvest the cells by centrifuging the overnight culture at 4000 × g for 10 minutes. Discard the supernatant.

3. Add **4 mL Cell Resuspension Solution (Buffer L1)** to resuspend the cells until homogeneous.

4. Add **4 mL Cell Lysis Solution (Buffer L2)**. Mix gently by inverting the tube until the mixture is thoroughly homogenous. **Do not vortex**. Incubate at room temperature for 5 minutes. ***Note:** Do not allow lysis to proceed for more than 5 minutes.*

5. Add **4 mL Neutralization Solution (Buffer L3)** Mix immediately by inverting the tube until the mixture is thoroughly homogeneous. **Do not vortex**.

6. Centrifuge the mixture at >12,000 × g for 10 minutes at room temperature. ***Note:** If the pellet does not adhere to the bottom of the tube, incubate the tube at room temperature for 5 minutes to allow the lysate and*

gelatinous pellet to separate. Pipet the clear lysate into another tube and centrifuge at >12,000 × g at room temperature for 5 minutes to remove any remaining cellular debris.

Binding and Washing DNA

1. Load the supernatant from step 6 (Preparing Cell Lysate) onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
2. Wash the column **twice** with **10 mL Wash Buffer (Buffer WB)**. Allow the solution in the column to drain by gravity flow after each wash. Discard the flow-through.

Eluting and Precipitating DNA

1. Place a sterile 15 mL centrifuge tube (elution tube) under the column.
2. Add **5 mL Elution Buffer (Buffer EB)** to the column to elute the DNA. Allow the solution to drain by gravity flow. Do not force out any remaining solution. The elution tube contains the purified DNA. Discard the column.
3. Add **3.5 mL isopropanol** to the elution tube. Mix well.
4. Centrifuge the tube at >15,000 × g for 30 minutes at 4°

C. Carefully remove and discard the supernatant.

5. Resuspend the pellet in **3 mL 70% ethanol**.

6. Centrifuge the tube at >15,000 × g for 5 minutes at 4°C. Carefully remove and discard the supernatant.

7. Air-dry the pellet for 10 minutes.

8. Resuspend the DNA pellet in **200 µL TE Buffer (TE)**. **For low copy number plasmids, use 100 µL of TE Buffer.** ***Note**: Occasionally, insoluble particles may be present which do not influence the quality of the DNA. To remove these insoluble particles, centrifuge the DNA solution at high speed for 1 minute at room temperature. Transfer the supernatant (DNA sample) into a fresh tube.*

9. Store the purified DNA at 4°C for immediate use or store at –20°C for long-term storage.

GeneBond™ MaxiPrep Flow Chart

Equilibrate column

30 mL Equilibration Buffer (Buffer EQ)

Prepare Cell Lysate

Harvest Cells

4, 000 x *g* , 10 min

10 mL Cell Resuspension Solution (Buffer L1)

10 mL Cell Lysis Solution (Buffer L2)

10 mL Neutralization Solution (Buffer L3)

>12,000 x *g* , 10 min

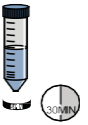
Bind and wash DNA

Load Column with lysate

60 mL Wash Buffer (Buffer WB)

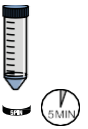
Elute and precipitate DNA

15 mL Elution Buffer (Buffer EB)



10.5 mL Isopropanol

>15,000 x *g* , 30 min



5 mL 70% Ethanol

>15,000 x *g* , 5 min

200 µL TE Buffer

General Protocol for MaxiPrep

The GeneBond™ MaxiPrep Plasmid Purification Kits allows you to purify 500 µg of plasmid DNA from 100-200 mL overnight *E. coli* cultures in ~3 hours when cloning high copy number plasmids.

Materials Needed

- Overnight culture of transformed *E.coli* cells (page 5)
- Isopropanol
- 70% ethanol
- Sterile, microcentrifuge tubes
- Tubes or centrifuge bottles for harvesting cells
- 50 mL centrifuge tubes (elution tubes)
- Centrifuge with temperature control at 4°C

Equilibrating the Column

Add **30 mL Equilibration Buffer (Buffer EQ)** to the column. Allow the solution in the column to drain through gravity flow, proceed to the preparing cell lysate while the column is in the equilibration process.

Preparing Cell Lysate

1. For **high copy number plasmids**, use 100-200 mL of an overnight LB culture per sample. For **low copy**

number plasmids, use 250-500 mL of an overnight LB culture per sample. ***Note**: *For using >200 mL of culture volume, add twice the amount of Cell Resuspension Solution (Buffer L1), Cell Lysis Solution (Buffer L2), and Neutralization Solution (Buffer L3) as directed in steps 3, 4, and 5, below, for best results.*

2. Harvest the cells by centrifuging the overnight culture at 4000 × g for 10 minutes. Discard the supernatant.

3. Add **10 mL Cell Resuspension Solution (Buffer L1)** to resuspend the cells until homogeneous.

4. Add **10 mL Cell Lysis Solution (Buffer L2)**. Mix gently by inverting the tube until the mixture is thoroughly homogenous. **Do not vortex**. Incubate at room temperature for 5 minutes. ***Note:** Do not allow lysis to proceed for more than 5 minutes.*

5. Add **10 mL Neutralization Solution (Buffer L3)**. Mix immediately by inverting the tube until the mixture is thoroughly homogeneous. **Do not vortex**.

6. Centrifuge the mixture at >12,000 × *g* for 10 minutes at room temperature. ***Note:** If the pellet does not adhere to the bottom of the tube, incubate the tube at room temperature for 5 minutes to allow the lysate and gelatinous pellet to separate. Pipet the clear lysate into another tube and centrifuge at >12,000 × g at room*

temperature for 5 minutes to remove any remaining cellular debris.

Binding and Washing DNA

1. Load the supernatant from step 6 (Preparing Cell Lysate) onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
2. Wash the column with **30 mL Wash Buffer (Buffer WB) Twice**. Allow the solution in the column to drain by gravity flow after each wash. Discard the flow-through.

Eluting and Precipitating DNA

1. Place a sterile 50 mL centrifuge tube (elution tube) under the column.
2. Add **15 mL Elution Buffer (Buffer EB)** to the column to elute the DNA. Allow the solution to drain by gravity flow. Do not force out any remaining solution. **The elution tube contains the purified DNA**. Discard the column.
3. Add **10.5 mL isopropanol** to the elution tube. Mix well.
4. Centrifuge the elution tube at >15,000 × *g* for 30 minutes at 4°C. Carefully remove and discard the supernatant.
5. Resuspend the pellet in **5 mL 70% ethanol**.

6. Centrifuge the tube at >15,000 × *g* for 5 minutes at 4°C. Carefully remove and discard the supernatant.

7. Air-dry the pellet for 10 minutes.

8. Resuspend the DNA pellet in **500 µL TE Buffer (TE)**. **For low copy number plasmids, use 200 µL of TE Buffer.** ***Note***: *Occasionally, insoluble particles may be present which do not influence the quality of the DNA. To remove these insoluble particles, centrifuge the DNA solution at high speed for 1 minute at room temperature. Transfer the supernatant (DNA sample) into a fresh tube*.

9. Store the purified DNA at 4°C for immediate use or store at –20°C for long-term storage.

Estimating DNA Yield and Quality

Determine the quantity and quality of the purified DNA using UV absorbance at 260 nm.

UV Absorbance

1. Prepare a dilution of the DNA solution in 10 mM Tris-HCl, pH 7.5. Mix well.
2. Measure the absorbance at 260 nm (A260) of the dilution in a spectrophotometer (using a cuvette with an optical path length of 1 cm) blanked against 10 mM Tris-HCl, pH 7.5
3. Calculate the concentration of DNA using the formula: DNA (µg/mL) = A_{260} × 50 × dilution factor

For DNA, A260 = 1 for a 50 mg/mL solution measured in a cuvette with an optical path length of 1 cm.

DNA Quality

Typically, DNA isolated using the GeneBond™ MidiPrep/ MaxiPrep Plasmid Purification Kits has an A260/A280 ratio >1.80 when samples are diluted in Tris-HCl pH 7.5, indicating that the DNA is substantially free of proteins that could interfere with downstream applications. The absence of contaminating RNA may be confirmed by agarose gel electrophoresis.

TROUBLESHOOTING

Q: PELLET/DEBRIS IS TRANSFERRED ONTO COLUMN

- ⇨ Pellet is viscous and does not adhere to tube: After centrifuging the lysate, allow the tube sit for 5 minutes to separate the clear lysate from the pellet (the pellet may be floating). Remove the clear lysate to a fresh tube and centrifuge again to remove any remaining debris. .
- ⇨ Using a high volume of culture: Use the recommended culture volumes. If you are using higher culture volumes than the recommended volume, double the volumes of the Cell Resuspension Solution (Buffer L1), Cell Lysis Solution (Buffer L2), and Neutralization Solution (Buffer L3) as designated in the protocol.

Q: LOW PLASMID DNA YIELD

- ⇨ Buffers not stored correctly: Store Cell Lysis Solution (Buffer L2) and Equilibration Buffer (Buffer EQ) at room temperature.
- ⇨ Lysate centrifuged at 4°C: Make sure that the rotor and the centrifuge are at room temperature for the lysate centrifugation step. If centrifugation at 4°C is unavoidable, transfer the cleared lysate to a new tube and warm it to room temperature in a water bath before loading it onto the column.

- ⇨ Low copy number plasmid: Increase the volume of starting culture. Carefully remove all medium before resuspending cells. Doubling the volumes of the Cell Resuspension Solution (Buffer L1) , Cell Lysis Solution (Buffer L2) and Neutralization Solution (Buffer L3) may increase plasmid yield and quality.
- ⇨ Lysate at improper pH or salt concentration to bind column: Make sure that the correct volume of Neutralization Solution (Buffer L3) is added when neutralizing the lysate.
- ⇨ Plasmid DNA pellet over-dried: Do not dry the DNA pellet with a vacuum system.

Q: SLOW COLUMN FLOW

- ⇨ Column clogged: Pipet the lysate supernatant onto the column. Do not pour the lysate onto the column, as some of the precipitate could enter the column.

Q: GENOMIC DNA CONTAMINATION

- ⇨ Genomic DNA sheared during handling: Gently invert tubes to mix after adding buffers. **Do not vortex** as it can shear genomic DNA.

Q: ADDITIONAL PLASMID FORMS PRESENT

- ⇨ Plasmid DNA permanently denatured (band migrating faster than supercoiled DNA) Incubate the lysate at room

temperature for no longer than 5 minutes.

Q: RNA CONTAMINATION

- ⇨ Lysate at improper pH, salt concentration, or temperature: Carefully remove all medium before resuspending cells. Make sure not to add an excess of Neutralization Solution (Buffer L3) when neutralizing the lysate. Do not warm the lysate above room temperature during while centrifuging.
- ⇨ Lysate left on column too long: Once the lysate is loaded onto the column, avoid delays in processing.
- ⇨ Lysate droplets remained on walls of column at elution: Wash droplets of lysate from the walls of the column with the Wash Buffer.

Manufactured and distributed by
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