PureLink™ Fast Low Endotoxin Midi Plasmid Purification Kit

Catalog Numbers A35892 and A36227

Pub. No. MAN0017146 **Rev.** B.0

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

Product description

Product information

The Invitrogen PureLink™ Fast Low Endotoxin Midi Plasmid Purification Kit features a column-based method for the purification of up to 400 µg of high-quality plasmid DNA in less than 30 minutes. The eluted plasmid DNA is ready for immediate use, avoiding the need for subsequent precipitation steps.

PureLink™ Fast technology uses a modified alkaline lysis method and features novel binding chemistry that yields highly concentrated plasmid DNA (up to 3 µg/µL). In addition, the wash regimen has been optimized to ensure the plasmid DNA is low endotoxin and free of salt, protein, and RNA. The result is plasmid DNA suitable for transfection, restriction endonuclease digestion, *in vitro* transcription, PCR amplification, and DNA sequencing.

As an added convenience, the PureLink™ Fast Low Endotoxin Midi Plasmid Purification Kit contains colored buffers that permit error-free visualization of complete bacterial cell lysis and neutralization.

Product specifications

Specifications	Midiprep kit
DNA purity	Ultrapure, low endotoxin (<1 EU/µg), typical $A_{260/280}$ ≥1.8 and $A_{260/230}$ ≥2.0,
Plasmid DNA yield[1]	Up to 400 µg
Plasmid DNA size	Up to 25 kb
Elution volume	≥200 µL of elution buffer or DNase free water
Processing time	30 minutes

[1] Actual yield is dependent on the plasmid copy number, culture growth conditions, and strain of *E. coli* utilized

Contents and storage

Reagents provided in the kit are sufficient for 25 (Cat. No. A35892) and 50 (Cat. No. A36227) midipreps.

Table 1 Components of PureLink™ Fast Low Endotoxin Midi Plasmid Purification Kit.

Component	Cat. No. A35892	Cat. No. A36227	Storage temp.
Resuspension buffer[1] (red)	210 mL	410 mL	4°C
Lysis buffer[2] (blue)	210 mL	410 mL	Room temp.
Precipitation buffer (yellow)	210 mL	410 mL	
Binding buffer	210 mL	410 mL	
Wash buffer 1	20 mL	55 mL	
Wash buffer 2 (concentrate)	10 mL	23 mL	
Elution buffer	6 mL	12 mL	
Column assembly[3]	25 columns	50 columns	
Syringe filters	25 filters	50 filters	
Syringe plungers	25 plungers	50 plungers	
Collection tubes	25 tubes	50 tubes	
Quick reference card	1 card	1 card	

[1] Resuspension Buffer contains RNase A (100 µg/mL) and is stable at room temperature without loss in RNase activity, however, for long-term storage the product should be stored at 4–8° C.

[2] Caution: Lysis Buffer contains NaOH. Use proper safety precautions.

[3] 15 mL Conical Reservoir and 50 mL Reservoir are pre-assembled as a single unit.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Microcentrifuge capable of reaching ≥10,000 × *g* at room temperature	MLS
Thermo Scientific™ FastVac™ Vacuum Manifold or equivalent	Cat. No. A35899
Vacuum source capable of 400 mm Hg pressure at the vacuum manifold	MLS
Pipette for 100 µL to 800 µL	MLS
Sterile, endotoxin-free/pyrogen-free plastic pipette tips	MLS
Tubes with a minimum volume of 50 mL	MLS
1.5 mL centrifuge tubes	MLS
95–100% Ethanol (EtOH)	MLS

- The vacuum pump should be a single or double-staged unit capable of producing up to 400 mm Hg pressure at the vacuum manifold.
- The Lysis Buffer and Binding Buffer may have precipitant. If this occurs, dissolve the precipitate by incubating the bottles at 30–37°C for 10–20 minutes and mix by inversion. Do not microwave.
- Elution Buffer contains 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If required, pure water can also be used to elute the plasmid DNA.
- DNA yield can be increased by pre-warming the Elution Buffer to 50°C and/or increasing the incubation period up to 5 minutes prior to centrifugation.
- For low copy number plasmids or if higher concentration is desired, plasmid DNA can be eluted in as little as 100 µL.

Before you begin

- Add 38 mL of 95–100% ethanol to the 10 mL Wash Buffer 2 before use.

Midiprep plasmid isolation protocol

Procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.

Prepare the cell lysate

1 Pellet and resuspend cells

a. Centrifuge up to 50 mL of bacterial culture at ≥3,500 × *g* for 10 minutes to pellet the cells.

b. Discard supernatant.

c. Add 8 mL of Resuspension Buffer (red) to the bacterial cell pellet and resuspend completely by vortexing or pipetting.

2 Add lysis buffer

a. Add 8 mL of Lysis Buffer (blue) and immediately mix by gently inverting the tube 6 times.

Do not vortex.

b. Incubate at room temperature for 3 minutes.

Cells are completely lysed when the solution appears purple and viscous.

IMPORTANT! Do not allow lysis to proceed for more than 3 minutes. Excessive lysis can result in denatured plasmid DNA.

3 Add precipitation buffer

a. Add 8 mL of Precipitation Buffer (yellow) and mix gently 6 times (or until homogenous) by inversion.

Do not vortex.

The sample will turn yellow when the neutralization is complete and a yellowish precipitate will form.

b. Ensure the pre-assembled plug is screwed in tightly to the Luer-Lok at the bottom of the Syringe Filter.

3	Add precipitation buffer *(continued)*	c. Place the syringe filter upright in a tube rack and load the lysate into the Syringe Filter. d. Wait 5 minutes for the precipitate to float to the top.
4	Filter the lysate	a. Remove the Luer-Lok plug from the bottom of the syringe and place it into a clean 50 mL conical tube. b. Place the plunger in the syringe and push the solution through the Syringe Filter until approximately 20 mL of clarified lysate is recovered. **IMPORTANT!** Do not use excess pressure on the plunger. c. Save the clarified lysate.

Bind the DNA

1	Add binding buffer	Add 8 mL of Binding Buffer to the clarified lysate from the previous step and mix thoroughly by inverting the capped tube 10 times.
2	Bind DNA to column	a. Ensure the connections of the column assembly are finger-tight, then place onto a vacuum manifold. b. Add the mixture from the previous step into the column assembly and turn on the vacuum until all the liquid has passed through the column. **Note:** For optimal performance, the vacuum pump should apply at least 400 mm Hg pressure. If less pressure is applied, centrifuge the column prior to washing to remove residual lysate remaining in the matrix.

Recover the DNA

1	Wash the DNA	a. Unscrew the purple Luer-Lok cap from the top of the column and discard the reservoirs. b. With the vacuum off, add 800 µL of Wash Buffer 1 to the column. c. Turn on the vacuum until all the liquid has passed through the column. d. With the vacuum off, add 800 µL of Wash Buffer 2 (with EtOH added) to the column. e. Turn on the vacuum until all the liquid has passed through the column. f. Repeat steps d and e.
2	Elute the DNA	a. Place the column in a collection tube and transfer to a microcentrifuge. b. Centrifuge at ≥10,000 × *g* for 1 minute to remove any residual wash buffer. c. Transfer the column into a clean 1.5 mL tube (not provided). d. Add 200 µL of Elution Buffer directly to the column matrix and incubate at room temperature for 2 minutes. e. Centrifuge at ≥10,000 × *g* for 1 minute in a microcentrifuge. f. Store the eluted plasmid DNA at ≤ –20°C.

Troubleshooting

Observation	Possible cause	Recommended action
Low DNA yield	Culture growth conditions	Poor aeration of culture. The optimal culture volume to air volume ratio is 1:4 or higher. For best aeration, use baffled culture flasks, or a vented or gas-permeable seal on the culture vessel.
		The culture was overgrown, undergrown, contaminated, or antibiotics were omitted from the growth medium. Use a fresh culture for optimal performance. An OD_{600} of 2 to 3.5 is the optimal optical density for bacterial culture.
	Cell density is too high	Too much culture used. Lysis and neutralization will be incomplete and the Syringe Filter may clog during filtration. More culture does not always equal more plasmid. Incomplete lysis and neutralization are two of the most common causes of failed plasmid preps and both are caused by too much culture being used.
		Incomplete lysis: After addition of Lysis Buffer, the solution should change from opaque pink to a clear viscous purple, indicating complete lysis. Different *E. coli* strains often require different growth conditions and may vary in their susceptibility to alkaline lysis.
		Incomplete neutralization: The solution should not be viscous following neutralization and the yellowish precipitate should appear fluffy and readily float to the surface. Make sure the neutralization is complete prior to filtration. Invert the tube an additional 2–3 times after the sample turns yellow following the addition of Precipitation Buffer.
	Lysate Clarification	Less than 20 mL of cleared lysate was recovered from the Syringe Filter. For optimal performance, add 8 mL of Binding Buffer to approximately 20 mL of clarified lysate.
	Lysis Buffer and Binding Buffer precipitated	Both buffers may have precipitated during shipping. To completely resuspend the buffers, incubate the bottles at 30–37°C for 10 minutes and mix by inversion. DO NOT MICROWAVE.
	Wash buffer	Ensure that ethanol has been added to Wash 2.
		Ensure that the bottle cap is screwed on tightly after each use to prevent evaporation of the ethanol.
	DNA elution	Incomplete elution: For large size plasmids (>10 kb), add Elution Buffer and incubate the column for 5–10 minutes before centrifugation. Also, pre-warm the Elution Buffer to 50°C prior to elution.
Low DNA quality	DNA does not perform well	Incomplete neutralization: Incomplete neutralization generates poor quality supernatant. Ensure that neutralization is complete by inverting the sample an additional 2-3 times after the addition of precipitation buffer and extending the incubation.
		Ethanol contamination in eluate. Centrifuge the column to dry the matrix, as indicated in the protocol prior to adding the Elution Buffer.
	RNA in eluate	Ensure that the resuspension buffer has been stored at 4°C. RNase A can be purchased separately if necessary.
	Genomic DNA in eluate	Improper handling (Sample was vortexed or handled too roughly). Genomic DNA contamination is usually caused by excessive mechanical shearing during the lysis and neutralization steps. Also, prolonged lysis or incomplete mixing of lysis or neutralization buffers may contribute to genomic DNA contamination in your sample.
		Overgrown culture. Overgrown or old cultures may contain more genomic DNA contamination than fresh cultures.
Slow flow rate through the column in presence of vacuum	Precipitate in sample	Excess pressure on the plunger allowed precipitate to flow into the clarified sample. Centrifuge the sample for 5 minutes at 1000 × *g* and decant the clarified lysate from the pellet.

Limited product warranty

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Revision	Date	Description
B.0	11-September-2017	Update to vacuum manifold name.
A.0	07-August-2017	New product

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