PureLink™ Fast Low Endotoxin Midi Plasmid Purification Kit

QUICK REFERENCE

invitrogen

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Package Contents

Catalog Numbers:	Amount:
A35892	25 preps
A36227	50 preps

Storage Conditions

- Upon receipt, store Resuspension Buffer at 4°C, and all the other components at room temperature

Required Materials

- Thermo Scientific™ FastVac™ Vacuum Manifold with vacuum source capable 400 mm Hg pressure at the vacuum manifold
- Microcentrifuge capable of reaching ≥10,000 × g at room temperature
- Tubes with a minimum volume of 50 mL
- 1.5 mL centrifuge tubes
- Pipette for 100 to 800 µL
- 95–100% Ethanol

Timing

Bacterial culture: overnight
Purification: 30 minutes

Selection Guide

Go online to view related products:
PureLink™ Nucleic Acid Purification Kit

Product Description

- The PureLink™ Fast Low Endotoxin Midi Plasmid Purification Kit enables isolation of high quality, low endotoxin (<1 EU/ug), plasmid DNA ready for immediate use, avoiding the need for subsequent precipitation steps
- Isolate up to 400 ug of high quality, ultrapure plasmid DNA from 50 mL of bacterial culture
- DNA is free of RNA, salt, and protein, making it ideal for transfection, restriction endonuclease digestion, *in-vitro* transcription, PCR amplification, and DNA sequencing
- Colored buffers that permit error-free visualization of complete bacterial cell lysis and neutralization

Important Guidelines

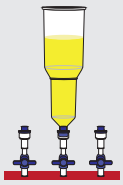
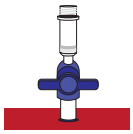
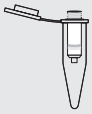
- The Lysis Buffer and Binding Buffer may have precipitant. If this occurs, dissolve the precipitate by incubating the bottles at 30–37°C for 10–20 minutes and mix by inversion
- Elution Buffer contains 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If required, pure water can also be used to elute the plasmid 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

Online Resources

Visit our product page for additional information and protocols. For support, visit **thermofisher.com/support**.

Midiprep plasmid isolation protocol

Before first use of the kit, add 38 mL of 95–100% ethanol to the 10 mL of Wash Buffer 2. Mark the label to indicate that ethanol is added.

	Steps		Procedure Details
1	**Pellet the cells**		Sediment cells by centrifugation for **10 minutes** at **≥3,500 x *g***, then discard the supernatant.
2	**Add Resuspension Buffer**		Add **8 mL** of Resuspension Buffer (red) to the cell pellet and resuspend by vortexing or pipetting.
3	**Add Lysis Buffer**		Add **8 mL** of Lysis Buffer (blue) and mix by inverting 6 times. Do not vortex. Incubate at room temperature for **3 minutes.** Lysis is complete when the mixture turns dark purple and viscous.
4	**Add Precipitation Buffer**		Add **8 mL** of Precipitation Buffer (yellow) and mix by inverting 6 times. Do not vortex. The sample will turn yellow when neutralization is complete.
5	**Load the lysate**		Load the lysate into the syringe filter and wait **5 minutes** until the precipitate has floated to the top.
6	**Filter the lysate**		Remove the lock and filter the lysate into a fresh 50 mL tube. Do not use excess pressure. Save this clarified lysate.
7	**Add Binding Buffer**		Add **8 mL** of Binding Buffer to the clarified lysate and mix by inverting 10 times.
8	**Bind DNA to the column**		Add the mixture into the column assembly and turn on the vacuum until all the liquid has passed through the column.
9	**Wash DNA**		Unscrew the purple Luer-Lok cap from the top of the column and discard the reservoirs. Wash with **800 µL** of Wash Buffer 1 (once), and then with **800 µL** of Wash Buffer 2 (twice), using the vacuum manifold. Turn off the vacuum between washes.
10	**Elute DNA**		Transfer column to a 1.5 mL collection tube and centrifuge at **≥10,000 x *g*** for 1 minute to remove any residual Wash Buffer.Transfer the column to a fresh 1.5 mL tube. Add **200 µL** of Elution Buffer and incubate for 2 minutes, then centrifuge at **≥10,000 x *g*** for 1 minute to elute the DNA.

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