

# GENELUTE ENDOTOXIN-FREE PLASMID MAXIPREP KIT

Bacterial culture



Pure Endotoxin free Plasmid DNA

## 1 Harvest & lyse bacteria

- Pellet cells from up to 130 ml overnight culture at 4,000-5,000 x g, 10 min. Discard supernatant.
- Add 4.8 ml of Resuspension Solution (S1). Resuspend the pellet until homogeneous.
- Add 4.8 ml of Lysis Solution (S2). Invert gently to mix. Incubate at room temperature for  $\leq 5$  min.

\*Prior to first time use, be sure to add RNase A to the Resuspension Solution.

## 2 Prepare cleared lysate

- Add 3.2 ml of Neutralization Solution. Mix thoroughly by gentle inversion.
- Pellet debris at  $\geq 15,000$  x g, 15 min. Transfer cleared lysate into a 15 ml tube.

## 3 Remove endotoxins

- Add 1.2 ml of Endotoxin Removal Solution. Mix thoroughly. Chill on ice for  $\geq 10$  min.
- Warm in a 37°C water bath for 5 min. Spin in a swinging bucket rotor at 3,000-5,000 x g, 5 min.
- Transfer the clear upper phase to a fresh tube and discard the blue lower phase. Repeat the endotoxin removal steps once.

## 4 Bind plasmid DNA to column

- Add 3.2 ml of DNA Binding Solution to the upper phase. Mix thoroughly.
- Transfer into Maxi Binding Column in a collection tube.
- Spin in a swinging bucket rotor at 3,000-5,000 x g, 1-2 min. Discard flow through.

## 5 Wash to remove contaminants

- Optional Wash:*  
Add 8 ml of Optional Wash Solution (OWS) to column. Spin in a swinging bucket rotor at 3,000-5,000 x g, 2 min. Discard flow through.
- Add 15 ml of Wash Solution (WS) to column. Spin in a swinging bucket rotor at 3,000-5,000 x g, 5 min.

\*Prior to first time use, be sure to add ethanol to the Wash Solution Concentrate.

## 6 Elute purified plasmid DNA

- Transfer column to new collection tube.
- Add 5 ml of Endotoxin Free Water to column. Spin in a swinging bucket rotor at 3,000-5,000 x g, 3-5 min.



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Problem	Reason	Solution
Poor or low Plasmid DNA recovery	Binding columns were spun in a fixed angle rotor, or with insufficient g-force	Binding columns must be spun in a swinging bucket rotor at 3,000-5,000 x g for liquids to pass through efficiently. Actual spin speed in RPM will depend on rotor size (see note at beginning of Procedure in the Technical Bulletin).
	Wash Solution is too concentrated	Confirm the Wash Solution concentrate was diluted with the specified volume of ethanol. Keep bottle tightly capped between uses to prevent evaporation.
	Number of cells is insufficient	Culture may be too old. Prepare a new culture. <b>OR</b> Confirm cell density. Grow culture to 2.5-3.0 OD <sub>600</sub> .
	Plasmid replication is poor	Confirm cells were grown in appropriate media under optimized conditions.
	Antibiotic activity is insufficient	Use fresh antibiotic for growth of overnight cultures. Most antibiotics are light sensitive and degrade during long term storage at 2-8 °C.
	Alkaline lysis is prolonged	Reduce the lysis time to 3 minutes or until the suspended cells form a clear viscous solution.
Absorbance of final product does not match actual quantity of plasmid	Precipitation of cell debris is incomplete	Reduce the initial volume of cell culture.
	Lysis is incomplete	Reduce the initial volume of cell culture or increase the lysis time while monitoring the lysis visually.
	Plasmid DNA is contaminated with RNA; RNase A treatment is insufficient	Confirm that the RNase A Solution was added to the Resuspension Solution prior to first use. The RNase A Solution may degrade due to high temperatures (>65 °C) or prolonged storage (>6 months at room temperature).
Poor A <sub>260</sub> /A <sub>280</sub> ratio for the purified DNA	Plasmid DNA is contaminated with chromosomal DNA	Do not use cultures that have grown for more than 24 hours or are in the cell death phase. Do not vortex or vigorously shake the cells during the lysis reaction or neutralization procedure.
	Background reading is high due to silica fines	Spin DNA sample at maximum speed for 1 minute; use supernatant to repeat absorbance readings.
The Endotoxin Removal Solution is in two phases	Purification is incomplete due to column overloading	Reduce the initial volume of cell culture.
	Storage temperature is higher than 25°C	Mix the solution briefly and incubate on ice for >10 minutes before use. Solution will be clear, blue and homogeneous (in one phase).
Residual endotoxin level >0.1 EU/µg DNA	Culture overgrown or too much culture used	Grow culture 12-16 hr. with vigorous shaking. Do not exceed the recommended maximum culture volume (130 ml).
	Endotoxin-enriched lower (blue) phase is carried over	Avoid pipetting any part of the blue lower phase when transferring the clear upper phase. Perform the Optional Wash step to remove endotoxins that may have been carried over.
Poor performance in downstream enzymatic applications	Purification is incomplete	Salts in one or more of the solutions may have precipitated. Heat the solution at 65 °C until dissolved. Cool to room temperature prior to use.
	DNA concentration is too low	Precipitate the DNA with ethanol, then resuspend the DNA in a smaller volume of endotoxin-free water. <b>OR</b> Elute silica-bound DNA with less endotoxin-free water. Note that using less water may reduce the overall recovery.
	DNA was prepared from EndA <sup>+</sup> strains	The Optional Wash Step must be included when recovering DNA from EndA <sup>+</sup> strains.
Additional band migrating ahead of supercoiled plasmid during electrophoresis	The final plasmid DNA eluate contains too much salt	Precipitate the DNA using ethanol. Dry pellet. Redissolve in endotoxin-free water.
	The column contains residual ethanol from diluted Wash Solution.	Re-centrifuge the column for 1 minute after washing to remove any residual Wash Solution.
	A portion of the plasmid DNA is permanently denatured	Do not allow the lysis reaction to exceed 5 minutes. Note that nicked (covalently open) double-stranded plasmid DNA runs slower than supercoiled DNA during electrophoresis.