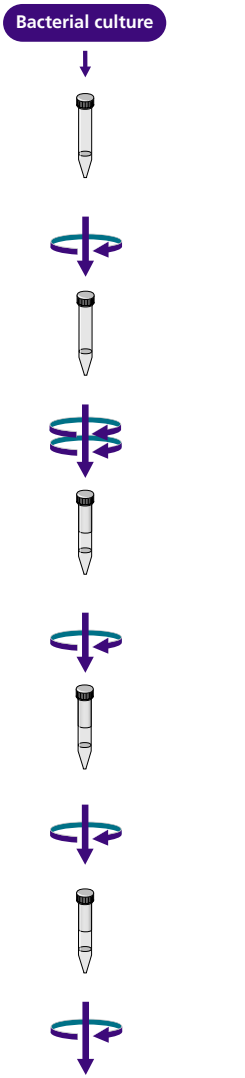
PLED 35

GENELUTE ENDOTOXIN-FREE PLASMID MIDIPREP KIT



1 Harvest & lyse bacteria

- ❑ Pellet cells from up to 40 ml overnight culture at 3,000-5,000 x g, 10 min. Discard supernatant.
- ❑ Add 1.2 ml of Resuspension Solution. Resuspend the pellet until homogeneous.
- ❑ Add 1.2 ml of Lysis Solution. Invert gently to mix. Incubate at room temperature for ≤ 5 min.

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

2 Prepare cleared lysate

- ❑ Add 0.8 ml of Neutralization Solution. Mix thoroughly by gentle inversion.
- ❑ Pellet debris at ≥ 15,000 x g, 15 min. Transfer cleared lysate into a 15 ml tube.

3 Remove endotoxins

- ❑ Add 300 µl of Endotoxin Removal Solution. Mix thoroughly. Chill on ice for ≥ 5 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 0.8 ml of DNA Binding Solution to the upper phase. Mix thoroughly.
- ❑ Transfer into Midi 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 2 ml of Optional Wash Solution to column. Spin in a swinging bucket rotor at 3,000-5,000 x g, 2 min. Discard flow through.
- ❑ Add 3 ml of Wash Solution 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 1 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 Plasmid DNA yield	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* in steps 6-9 for liquids to pass through efficiently. Actual spin speed in RPM will depend upon rotor size (see note at beginning of the Procedure Section).
	Wash Solution is too concentrated	Confirm that 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. • Confirm cell density. Grow culture to 2.5-3.0 OD_{600}.
	Plasmid replication is poor	Confirm cells were grown in appropriate media under optimized conditions. If low copy plasmid, use the Maxi kit and increase the culture volume accordingly.
	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 time for cell lysis to 3 minutes or until the suspended cells form a clear viscous solution after inversion with the Lysis Solution.
	Residual supernatant from cell media	After initial centrifugation step of cell culture, remove supernatant and centrifuge a second time to remove any remaining supernatant.
	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.
Less plasmid DNA than expected from absorbance readings or poor A_{260}/A_{280} ratios	Wash Solution is diluted with ethanol containing impurities	Check the absorbance of ethanol between 250 and 300 nm. Do not use denatured ethanol with high absorbance. Traces of impurities may remain on binding column after washing. The impurities could show up in the eluate and may contribute to the absorbance in the final product.
	RNA contamination because RNase A treatment is insufficient	Confirm that RNase A was added to the Resuspension Solution prior to first use. RNase A may degrade due to high temperatures (>65°C) or prolonged storage (>6 months).
	Chromosomal DNA contamination due to shearing	Do not vortex or vigorously shake the cells during lysis or neutralization.
	Overgrown culture	Do not use cultures that have grown for more than 24 hours or are in the cell death phase.
	Purification is incomplete due to large amount of cells	Reduce the initial volume or the density of cell culture.
	Background reading is high due to silica fines	Spin DNA sample at maximum speed for 1 minute and use the supernatant for absorbance reading.
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 **Or** Elute silica-bound DNA with less endotoxin free water. Note that using less endotoxin free water may reduce the overall recovery.
	DNA was prepared from EndA+ strains	• The Optional Wash Step must be included when recovering DNA from EndA+ strains.
	Residual ethanol	Re-centrifuge the column for 1 minute after the washing to remove any residual Wash Solution.
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 (40 ml).
	Carry-over of the endotoxin-enriched lower (blue) phase	Avoid pipetting any part of the blue lower phase when transferring the clear upper phase. Perform the Optional Wash step.
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Endotoxin Removal Solution in two phases	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).
Additional band migrating ahead of supercoiled plasmid during electrophoresis	A portion of the plasmid DNA is permanently denatured	Do not allow the lysis reaction (step 3) to exceed 5 minutes. Note that nicked (covalently open) double-stranded plasmid DNA runs slower than supercoiled DNA during electrophoresis.