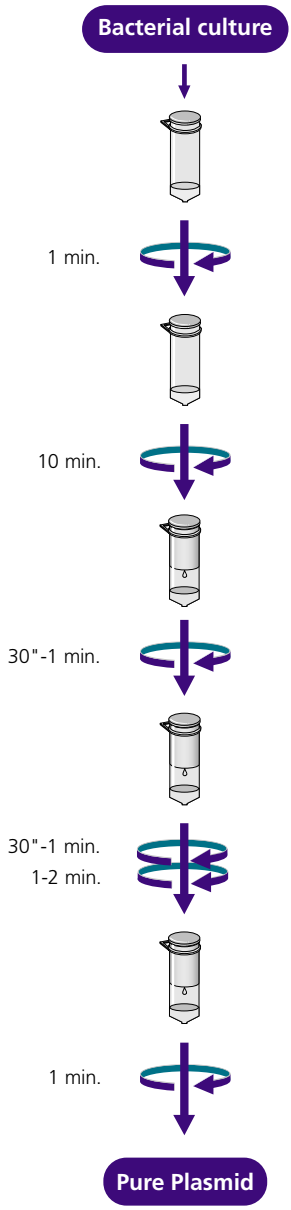
GENELUTE PLASMID MINI-PREP KIT



All spins at ≥ 12,000 x g, except as noted

1 Harvest & lyse bacteria

- ❑ Pellet cells from 1-5 ml overnight culture *1 min.* (1 ml from TB or 2xYT; 1-5 ml from LB medium) Discard supernatant.
- ❑ Resuspend cells in 200 µl resuspension solution. Pipet up and down or vortex.
- ❑ Add 200 µl of lysis solution. Invert gently to mix. Do not vortex. Allow to clear for ≤ 5 min.

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

2 Prepare cleared lysate

- ❑ Add 350 µl of neutralization solution (S3). Invert 4-6 times to mix.
- ❑ *Pellet debris 10 min. at max speed.*

3 Bind plasmid DNA to column

- ❑ Transfer cleared lysate into Mini Spin Column in a collection tube.
- ❑ *Spin 30"-1 min.* Discard flow-through.

4 Wash to remove contaminants

- ❑ *Optional (EndA+ strains only):* Add 500 µl optional wash solution to column. *Spin 30"-1 min.* Discard flow-through.
- ❑ Add 750 µl wash solution to column. *Spin 30"-1 min.* Discard flow-through.
- ❑ *Spin 1-2 min.* to dry column.

**Prior to first time use, be sure to add ethanol to the concentrated wash solution.*

5 Elute purified plasmid DNA

- ❑ Transfer column to new collection tube.
- ❑ Add 100 µl elution solution. *Spin 1 min.*

**If a more concentrated plasmid DNA prep is required, reduce the elution volume to a minimum of 50µl*

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Problem	Reason	Solution
Poor or Low Recovery	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.
	Insufficient number of cells	• Culture may be too old. Prepare a new culture. • Confirm cell density. Grow culture to 0. D600 = 2-3 for LB
	Poor plasmid replication	• Confirm cells were grown in correct media under correct conditions. • Confirm correct antibiotic was used to select the required strain of *E. coli* or other bacteria.
	Old antibiotic	Use fresh antibiotic for growth of overnight cultures. Most antibiotics are light-sensitive and degrade during long term storage at 2-8˚C.
	Prolonged alkaline lysis	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, remove supernatant; centrifuge a second time to remove any remaining supernatant.
	Incomplete precipitation of cell debris	Decrease culture volume.
	Incomplete lysis	Decrease culture volume or increase lysis time while visually monitoring.
Less plasmid than expected from absorbance reading or Poor A_{260} /A_{280} ratios	Incomplete purification due to high amount of DNA	Decrease culture volume.
	Background reading is high due to silica fines	Spin DNA sample(s) at maximum speed for 1 minute, use supernatant to repeat absorbance readings.
	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.
Chromosomal DNA contamination	Shearing of genomic DNA	Do not vortex or vigorously shake the cells during lysis or neutralization.
	Culture was overgrown	Do not use cultures that have grown for more than 24 hours or are in the cell death phase.
RNA contamination	Insufficient RNase A treatment	Confirm that 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 mo).
Additional forms of the plasmid present (single-stranded DNA)	Plasmid DNA is permanently denatured	There will be a second band ahead of supercoiled DNA during electrophoresis. 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.
Poor performance in downstream applications	Incomplete purification	Salts in one or more of the buffers may have precipitated out of solution. Heat the buffer at 65˚C until dissolved. Cool to room temperature prior to use.
	DNA concentration is too low	• Precipitate the DNA with alcohol, then resuspend DNA in a smaller volume of elution solution or water **Or**
		• Elute silica-bound DNA with less elution solution. Note that using less elution solution may reduce the overall recovery.
	DNA was prepared from EndA+ strains	The optional wash step must be included when recovering DNA from EndA+ strains.
	High salt content in the final plasmid DNA eluate	Precipitate the DNA using ethanol. Use an elution buffer other than elution solution. Elution solution contains EDTA, which may chelate divalent cations (e.g., Mg++) which are important co-factors for many enzymes.
	Residual ethanol from the diluted wash solution	Re-centrifuge the column for 1 minute after the wash step to remove any residual wash solution.