

Geneaid™ Midi Plasmid Kit EF Quick Protocol

For research use only

Catalogue Number

PIE02, PIE25

Instruction Manual Download

When using this product for the first time, or if you are unfamiliar with the procedure, please scan the QR code and download the complete instruction manual.

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*PW Buffer has been replaced with PMC Buffer. DO NOT USE leftover PW Buffer. **ONLY USE PMC Buffer with this kit.***

1. Harvesting

Transfer cultured bacterial cells to a 50 ml centrifuge tube then centrifuge at $\geq 3,000 \times g$ for 15 minutes at room temperature to form a cell pellet. Discard the supernatant completely. Repeat the Harvesting step as required for up to 200 ml of high-copy or 100-300 ml of low-copy bacterial cells using the same 50 ml centrifuge tube.

2. Equilibration

During centrifugation, place a **Plasmid Midi Column** in a new 50 ml centrifuge tube. Equilibrate the **Plasmid Maxi Column** by adding **5 ml of PEQ Buffer**. Allow the column to empty completely by gravity flow. Discard the flow-through and place the **Plasmid Midi Column** back in the 50 ml centrifuge tube. Set it aside for Step 7.

3. Resuspension

Add **4 ml of PM1 Buffer (make sure RNase A was added)** (Optional: Add 40 μ l of TrueBlue Lysis Buffer) to the 50 ml centrifuge tube containing the cell pellet. Resuspend the pellet by vortex, pipette or scraping the tube across the top of a 1.5 ml microcentrifuge tube rack until all traces of the pellet have been completely dissolved.

4. Cell Lysis

Add **4 ml of PM2 Buffer** to the resuspended sample then mix gently by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Let stand at room temperature for at least 2 minutes to ensure the lysate is homogeneous. Do not exceed 5 minutes.

5. Neutralization

Add 4 ml of PM3 Buffer. Mix by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Centrifuge at $\geq 3,000 \times g$ for 20 minutes at room temperature.

6. Endotoxin Removal

Transfer the supernatant to a clean 50 ml centrifuge tube. Add **1.2 ml of PER Buffer** then mix by inverting 5-10 times. Incubate on ice for 30 minutes.

NOTE: Following PER Buffer addition, the mixture will become cloudy.

7. DNA Binding

Transfer the cooled mixture to the equilibrated **Plasmid Midi Column**. Allow the column to empty completely by gravity flow. Discard the flow-through and place the **Plasmid Midi Column** back in the 50 ml centrifuge tube.

8. Wash

Wash the **Plasmid Midi Column** by adding **15 ml of PMC Buffer** and allow the column to empty completely by gravity flow then discard the flow-through.

9. Elution

Place the **Plasmid Midi Column** in a clean 50 ml centrifuge tube then add **8 ml of PEL Buffer** to elute the DNA by gravity flow. Discard the **Plasmid Midi Column** once it has emptied completely.

10. DNA Precipitation

Add **6 ml (0.75 volumes) of isopropanol** and mix completely by inverting. Centrifuge at $\geq 3,000 \times g$ for 20 minutes (preferably at $15,000 \times g$ for 30 minutes) at 4°C. Carefully remove the supernatant then wash the DNA pellet with **5 ml of 75% ethanol**. Centrifuge at $\geq 3,000 \times g$ for 5 minutes (preferably at $15,000 \times g$ for 10 minutes) at 4°C. Carefully remove the supernatant then air-dry the DNA pellet for 10 minutes. Once the DNA pellet is dry, add **2 ml (or a suitable volume) of TE or ddH₂O** then place the tube in a 60°C water bath for 5-10 minutes to dissolve the DNA pellet.

Geneaid™ Midi Plasmid Kit Components

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Component	PI002/PIE02	PI025/PIE25
PM1 Buffer ¹	10 ml	110 ml
PM2 Buffer ²	10 ml	110 ml
PM3 Buffer	10 ml	110 ml
PER Buffer (PIE02, PIE25 Only)	4 ml	40 ml
PEQ Buffer	12 ml	130 ml
PMC Buffer	35 ml	125 ml x 1 260 ml x 1
PEL Buffer	25 ml	220 ml
RNase A (50 mg/ml)	Added	200 µl
Plasmid Midi Columns	2	25
TrueBlue Lysis Buffer	150 µl	1.5 ml

¹For PIE25 add provided RNase A to PM1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. PM1 Buffer and RNase A mixture should be stored at 2-8°C for up to 6 months. For PIE02 samples, RNase A was already added to PM1 Buffer.

²If precipitates have formed in PM2 Buffer, warm in a 37°C water bath, followed by gentle shaking to dissolve.

Geneaid™ Midi Plasmid Kit Functional Test Data

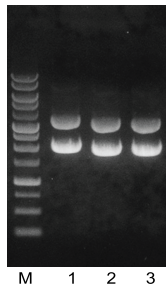


Figure 1. Plasmid DNA from a 100 ml overnight *E. coli* (DH5α) culture, containing plasmid pBluescript (OD600 = 3.65) was purified using the Geneaid™ Midi Plasmid Kit. The purified supercoiled plasmid DNA was analyzed by electrophoresis on a 1% agarose gel.

M = Geneaid 1 Kb DNA Ladder

Test	DNA Conc.	260/280	260/230	Yield
1	332.1 µg/ml	1.87	2.22	332.1 µg
2	345.5 µg/ml	1.87	2.25	345.5 µg
3	339.2 µg/ml	1.87	2.24	339.2 µg

Storage

Dry at room temperature (15-25°C)