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.

Instruction Manual Download

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 ≥3,000 x g for 15 minutes at room temperature to form a cell pellet. Discard the supernatant completely. Repeat the harvesting step as required volume of cultured bacterial cells using the same 50 ml centrifuge tube following the recommended volume below:

Plasmid Type	Pellet Wet Weight	OD600 = 2	OD600 = 4	OD600 = 6
High-copy number	0.75 g	200 ml	100 ml	66 ml
Low-copy number	1.12 g	300 ml	150 ml	100 ml

NOTE: For a higher quality and yield, increase the PM1, PM2 and PM3 buffer volumes by 1.5 times when using more than 0.75 g of cultured bacterial pellet. In this case, additional buffers can be purchased from Geneaid.

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 µl 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 ≥3,000 x g for 20 minutes at room temperature.

6. Endotoxin Removal

Transfer the supernatant to a clean 50 ml centrifgue 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 ≥3,000 x g for 20 minutes (preferably at 15,000 x g for 30 minutes) at 4ºC. Carefully remove the supernatant then wash the DNA pellet with **5 ml of 75% ethanol.** Centrifuge at ≥3,000 x g for 5 minutes (preferably at 15,000 x g for 10 minutes) at 4ºC. 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 ddH_2O** then place the tube in a 60ºC water bath for 5-10 minutes to dissolve the DNA pellet.

Geneaid™ Midi Plasmid Kit Components

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

[1]For 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 2-8ºC for up to 6 months. For PIE02 samples, RNase A was already added to PM1 Buffer.

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

Geneaid™ Midi Plasmid Kit Functional Test Data

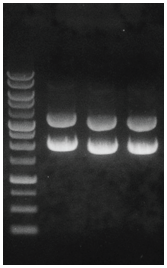

Figure 1. Plasmid DNA from a 100 ml overnight *E. coli* (DH5α) culture, containing plasmid pBluescript (OD600 = 3.65) was purified using the Geneaid™ Midi Plasmid Kit. The purified supercoiled plasmid DNA was analyzed by electrophoresis on a 1% agarose gel.
M = Geneaid 1 Kb DNA Ladder

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 (15-25ºC)