Geneaid™ Maxi Plasmid Kit EF Quick Protocol

For research use only

Catalogue Number

PME02, PME10, PME25

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 or a 250 ml centrifuge bottle 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 for up to 700 ml of high-copy or 350-1100 ml of low-copy bacterial cells using the same 50 ml centrifuge tube or 250 ml centrifuge bottle (See Recommended Culture Volume on page 2).

2. Equilibration

During centrifugation, place a **Plasmid Maxi Column** in a new 50 ml centrifuge tube. Equilibrate the **Plasmid Maxi Column** by adding **10 ml of PEQ Buffer**. Allow the column to empty completely by gravity flow. Discard the flow-through and place the **Plasmid Maxi Column** back in the 50 ml centrifuge tube. Set it aside for Step 7.

3. Resuspension

Add **10 ml of PM1 Buffer (make sure RNase A was added)** (Optional: Add 100 µl of TrueBlue Lysis Buffer) to the 50 ml centrifuge tube or 250 ml centrifuge bottle 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. NOTE: If the cell pellet is <2.6 g, transfer the resuspended sample to a new 50 ml centrifuge tube. If the cell pellet is >2.6 g, add 15 ml of PM1 Buffer and keep the resuspended sample in a 250 ml centrifuge bottle.

4. Cell Lysis

Add **10 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. NOTE: If the cell pellet is >2.6 g, add 15 ml of PM2 Buffer.

5. Neutralization

Add 10 ml of PM3 Buffer. Mix by inverting the tube 10 times. Do not vortex. Centrifuge at ≥3,000 x g for 20 minutes at room temperature. NOTE: If the cell pellet is >2.6 g, add 15 ml of PM3 Buffer.

6. Endotoxin Removal

Transfer the supernatant to a clean 50 ml centrifgue tube. Add **3 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 Maxi Column**. Allow the column to empty completely by gravity flow. Discard the flow-through and place the **Plasmid Maxi Column** back in the 50 ml centrifuge tube.

8. Wash

Wash the **Plasmid Maxi Column** by adding **30 ml of PMC Buffer** and allow the column to empty completely by gravity flow then discard the flow-through.

9. Elution

Place the **Plasmid Maxi Column** in a clean 50 ml centrifuge tube then add **12 ml of PEL Buffer** to elute the DNA by gravity flow. Discard the **Plasmid Maxi Column** once it has emptied completely.

10. DNA Precipitation

Add **9 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™ Maxi Plasmid Kit Components

Component	PME02	PME10	PME25
PM1 Buffer[1]	25 ml	110 ml	275 ml
TrueBlue Lysis Buffer	250 µl	1.5 ml	1.5 ml x 2
PER Buffer	8 ml	40 ml	100 ml
PM2 Buffer[2]	25 ml	110 ml	275 ml
PM3 Buffer	25 ml	110 ml	275 ml
PEQ Buffer	25 ml	130 ml	275 ml
PMC Buffer	65 ml	120 ml x 1 240 ml x 1	240 ml x 1 550 ml x 1
PEL Buffer	25 ml	130 ml	130 ml x 1 220 ml x 1
RNase A (50 mg/ml)	Added	200 µl	550 µl
Plasmid Maxi Columns	2	10	25

[1]For PME10, PME25 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 PME02 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™ Maxi Plasmid Kit Functional Test Data

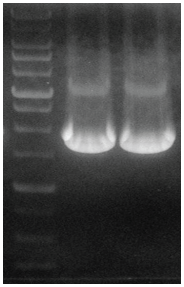

Figure 1. Plasmid DNA was extracted using the Geneaid™ Maxi Plasmid Kit. The purified supercoiled Plasmid DNA [300 ml overnight *E. coli* (DH5α) culture, containing a 3 kb plasmid pBluescript (A600 > 2 U/ml, OD600 = 4.0)], was analyzed by electrophoresis on a 1% agarose gel.
M = Geneaid 1 Kb DNA Ladder

Test	DNA Conc.	260/280	260/230	Yield
1	1012.2 µg/ml	1.87	2.25	1.0 mg
2	1015.1 µg/ml	1.87	2.29	1.0 mg

Recommended Culture Volume

Plasmid Type	Pellet Wet Weight	OD600 = 2	OD600 = 4	OD600 = 6
High-copy number	2.6 g	700 ml	350 ml	233 ml
Low-copy number	4 g	1100 ml	550 ml	366 ml

NOTE: For a higher yield, increase lysis buffer volumes by 1.5 times when using more than 2.6 g of cultured bacterial pellet. In this case, additional lysis buffer can be purchased from Geneaid.

Storage

Dry at room temperature (15-25ºC)