Presto™ Midi Plasmid Kit EF Quick Protocol

For research use only

Catalogue Number

PIFE02, PIFE25

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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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 for up to 100 ml of high-copy or 100-150 ml of low-copy cultured 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 Midi 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 then 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 cell pellet by vortex, pipette or scraping the tube across the top of a 1.5 ml microcentrifuge tube rack until all traces of the cell 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** and mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA.

6. Endotoxin Removal

Transfer all of the suspension to the **Filter Column (Yellow) in Collection Tube**. Centrifuge at 3,000 x g for 2 minutes at room temperature. NOTE: Invert PER Buffer bottle 3-5 times immediately prior to use.

Discard the **Filter Column (Yellow)** then add **1.2 ml of PER Buffer to the flow-through**. Seal the **Collection Tube with the provided Cap** 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

Following ice incubation, 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 **12 ml of PW 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** to the eluted DNA. Mix the tube 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.

Presto™ Midi Plasmid Kit Components

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Component	PIFE02	PIFE25
PM1 Buffer[1]	10 ml	110 ml
PM2 Buffer[2]	10 ml	110 ml
PM3 Buffer	10 ml	110 ml
TrueBlue Lysis Buffer	150 µl	1.5 ml
PER Buffer	4 ml	40 ml
PEQ Buffer	12 ml	130 ml
PW Buffer	30 ml	120 ml x 1 240 ml x 1
PEL Buffer	25 ml	220 ml
RNase A (50 mg/ml)	Added	200 µl
Filter Column (Yellow)	2	25
Collection Tube with Caps	2	25
Plasmid Midi Columns	2	25

[1]For PIFE25 add provided RNase A to PM1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. PM1 and RNase A mixture should be stored at 2-8ºC for up to 6 months. For PIFE02 samples, RNase A was already added to PM1 Buffer.

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

Presto™ Midi Plasmid Kit Functional Test Data

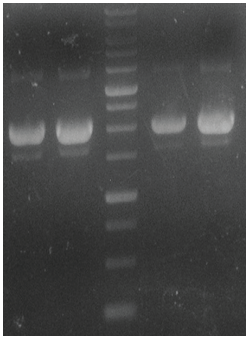

Figure 1. Plasmid DNA was extracted using both the Presto™ Midi Plasmid Kit (lane 1, 2) and the equivalent competitor's plasmid midi kit (lane 3, 4). The purified supercoiled plasmid DNA [50 ml and 100 ml overnight *E. coli* (DH5α) culture, containing a 3 kb plasmid pBluescript (A600 > 2 U/ml, OD600 = 3.8)], was used in *Eco*RI digestion and analyzed by electrophoresis on a 1% agarose gel. M = Geneaid 1 Kb DNA Ladder, Lane 1: Presto™ Midi Plasmid Kit (50 ml), Lane 2: Presto™ Midi Plasmid Kit (100 ml), Lane 3: Equivalent Competitor Kit (50 ml), Lane 4: Equivalent Competitor Kit (100 ml)

Kit	Test	260/280	260/230	Total Yield
Geneaid	50 ml	1.85	2.25	273.00 µg
	100 ml	1.87	2.14	409.20 µg
MN	50 ml	1.85	2.26	121.10 µg
	100 ml	1.87	2.33	289.80 µg

Related Plasmid DNA Purification Products

Product	Package Size	Catalogue Number
Presto™ Mini Plasmid Kit	100/300 preps	PDH100/300
Presto™ Midi Plasmid Kit	25 preps	PIF025
Presto™ Midi Plasmid Kit (Endotoxin Free)	25 preps	PIFE25
High-Speed Plasmid Mini Kit (10-50 Kb)	100/300 preps	PDL100/300
High-Speed Plasmid Advance Kit (50-100 ml)	25 preps	PA025
Geneaid™ Plasmid Mini Kit	40/100 preps	PAE040/100
Geneaid™ Plasmid Midi Kit	25 preps	PI025
Geneaid™ Plasmid Midi Kit (Endotoxin Free)	25 preps	PIE25
Presto™ Plasmid DNA Concentration Kit	250/500/1000 preps	PC0250/500/1000
Geneaid™ Plasmid Maxi Kit	10/25 preps	PM010/25
Geneaid™ Plasmid Maxi Kit (Endotoxin Free)	10/25 preps	PME10/25
Presto™ 96 Well Plasmid Kit	4/10 x 96 preps	96PDV04/10, 96PDC04/10