# 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.

*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 |
|---|---|---|---|
| High-copy number | 0.375 g | 100 ml | 50 ml |
| Low-copy number | 0.75 g | 150 ml | 100 ml |

## 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 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 by inverting the tube 10 times. Do not vortex.

## 6. Endotoxin Removal

Transfer the suspension to the Filter Column in Collection Tube. Centrifuge at 3,000 x g for 2 minutes at room temperature. Invert PER Buffer bottle 3-5 times immediately prior to use. Discard the Filter Column. Add 1.2 ml of PER Buffer to the flow-through. Seal the Collection Tube with the cap then mix by inverting 5-10 times. Incubate on ice for 30 minutes. 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** 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$_2$O** then place the tube in a 60ºC water bath for 5-10 minutes to dissolve the DNA pellet.

# Presto™ Midi Plasmid Kit Components

| 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 |
| PMC Buffer | 35 ml | 125 ml x 1<br>260 ml x 1 |
| PEL Buffer | 25 ml | 220 ml |
| RNase A (50 mg/ml) | Added | 200 µl |
| Filter Column | 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

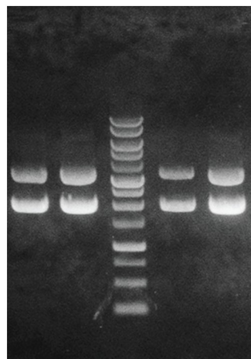


**Figure 1.** Plasmid DNA was extracted using both the Presto™ Midi Plasmid Kit (lane 1, 2) and the equivalent competitor's plasmid midi kit (lane 3, 4). The purified supercoiled plasmid DNA [50 ml and 100 ml overnight *E. coli* (DH5α) culture, containing a 3 kb plasmid pBluescript (A600 > 2 U/ml, OD600 = 3.8)], was 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)

| Brand | Test Volume | 260/280 | 260/230 | Yield |
|---|---|---|---|---|
| Geneaid | 50 ml | 1.85 | 2.20 | 252.2 µg |
| | 100 ml | 1.87 | 2.24 | 365.5 µg |
| MN | 50 ml | 1.85 | 2.26 | 121.1 µg |
| | 100 ml | 1.87 | 2.33 | 289.8 µg |

## Storage

Dry at room temperature (15 25ºC)