Presto™ Endotoxin Free Mini Plasmid Kit Quick Protocol

For research use only

Catalogue Number

PEH004, PEH100

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

1. Harvesting

Transfer **1.5 ml of cultured bacterial cells** (1-2 x 10^9 *E. coli* grown in LB medium) to a 1.5 ml microcentrifuge tube. Centrifuge at 14-16,000 x g for 1 minute at room temperature to form a cell pellet then discard the supernatant completely. Repeat the harvesting step as required for samples between 1.5-5.0 ml using the same 1.5 ml microcentrifuge tube.

2. Resuspension

Add **200 µl of PE1 Buffer (make sure RNase A was added)** (Optional: Add 2 µl of TrueBlue Lysis Buffer) to the 1.5 ml microcentrifuge tube containing the cell pellet. Resuspend the cell pellet completely by vortex or pipette until all traces of the cell pellet have been dissolved.

3. Cell Lysis

Add **200 µl of PE2 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.

4. Neutralization

Add **200 µl of PE3 Buffer** then mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Centrifuge at 14-16,000 x g for 5 minutes at room temperature. Transfer all of the clear supernatant to a new 1.5 ml microcentrifuge tube without disrupting the white precipitate.

5. Endotoxin Removal

Add **60 µl of PE4 Buffer** then vortex to mix. NOTE: PE4 Buffer is a viscous solution. To pipette PE4 Buffer, draw up and dispense slowly. The solution will become turbid after the addition of PE4 Buffer. Incubate the sample mixture on ice for 5 minutes then heat at 65ºC for 5 minutes. Centrifuge at 14-16,000 x g for 5 minutes. After centrifugation, the upper aqueous phase will be clear and the bottom phase will be red and viscous.

Transfer the upper aqueous phase to a new 1.5 ml microcentrifuge tube. DO NOT contact the red bottom phase as this contains endotoxin. Repeat the above extraction procedure one more time. Transfer the upper aqueous phase to a new 1.5 ml microcentrifuge tube. Centrifuge the tube at 14-16,000 x g for 5 minutes. If a small red pellet is present at the bottom of the tube, transfer the supernatant to a new 1.5 ml microcentrifuge tube without disturbing the red pellet.

6. DNA Binding

Add **350 µl of PE5 Buffer** to the sample and vortex to mix. Place a **PDH Column in a 2 ml Collection Tube**. Transfer **700 µl of the sample mixture** to the **PDH Column**. Centrifuge at 14-16,000 x g for 30 seconds at room temperature then discard the flow-through. Place the **PDH Column** back in the **2 ml Collection Tube**. Transfer the remaining sample mixture to the **PDH Column**. Centrifuge at 14-16,000 x g for 30 seconds at room temperature then discard the flow-through. Place the **PDH Column** back in the **2 ml Collection Tube**.

7. Wash

For Improved Downstream Sequencing Reactions

Add **400 µl of W1 Buffer** into the **PDH Column**. Centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through then place the **PDH Column** back in the 2 ml Collection Tube. Proceed with Wash Buffer addition.

For Standard Plasmid DNA Purification

Add **600 µl of Wash Buffer (make sure absolute ethanol was added)** into the **PDH Column**. Centrifuge at 14-16,000 x g for 30 seconds at room temperature. Discard the flow through then place the **PDH Column** back in the 2 ml Collection Tube. Centrifuge at 14-16,000 x g for 3 minutes at room temperature to dry the column matrix. Transfer the dried **PDH Column** to a new 1.5 ml microcentrifuge tube.

8. Elution

Add **50 µl of Elution Buffer**, TE or water into the **CENTER** of the column matrix. Let stand for at least 2 minutes to allow Elution Buffer, TE or water to be completely absorbed. Centrifuge at 14-16,000 x g for 2 minutes at room temperature to elute the purified DNA.

Presto™ Endotoxin Free Mini Plasmid Kit Components

Component	PEH004	PEH100
PE1 Buffer[1]	1 ml	25 ml
PE2 Buffer[2]	1 ml	25 ml
PE3 Buffer	1 ml	25 ml
PE4 Buffer	0.5 ml	12.5 ml
PE5 Buffer	1.5 ml	40 ml
TrueBlue Lysis Buffer	10 µl	250 µl
W1 Buffer	2 ml	45 ml
Wash Buffer[3] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)
Elution Buffer	1 ml	6 ml
RNase A (50 mg/ml)	Added	100 µl
PDH Columns	4	100
2 ml Collection Tubes	4	100

[1]For PEH100 add provided RNase A to PE1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. PE1 and RNase A mixture should be stored at 2-8ºC for up to 6 months. For PEH004 samples, RNase A was already added to PE1.

[2]If precipitates have formed in PE2 Buffer, warm the buffer in a 37ºC water bath, followed by gentle shaking to dissolve.

[3]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Storage

Dry at room temperature (15-25ºC).