Presto™ Mini Plasmid Kit Quick Protocol

For research use only

Catalogue Number

PDH004, PDH100, PDH300

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 1.5-7.0 ml using the same 1.5 ml microcentrifuge tube.

2. Resuspension

Add **200 µl of PD1 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 PD2 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 **300 µl of PD3 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 3 minutes at room temperature. If using >5 ml of bacterial cells, centrifuge at 16-20,000 x g for 5-8 minutes. During centrifugation, place a **PDH Column** in a 2 ml Collection Tube.

5. DNA Binding

Transfer all of the supernatant to the **PDH Column**. Use a narrow pipette tip to ensure the supernatant is completely transferred without disrupting the white precipitate. 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.

6. 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.

7. 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™ Mini Plasmid Kit Components

Component	PDH004	PDH100	PDH300
PD1 Buffer[1]	1 ml	25 ml	65 ml
PD2 Buffer[2]	1 ml	25 ml	75 ml
PD3 Buffer	1.5 ml	45 ml	100 ml
TrueBlue Lysis Buffer	10 µl	250 µl	650 µl
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer[3] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	1 ml	6 ml	30 ml
RNase A (50 mg/ml)	Added	100 µl	260 µl
PDH Columns	4	100	300
2 ml Collection Tubes	4	100	300

[1]For PDH100 and PDH300 add provided RNase A to PD1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. PD1 and RNase A mixture should be stored at 2-8ºC for up to 6 months. For PDH004 samples, RNase A was already added to PD1.

[2]If precipitates have formed in PD2 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.

Presto™ Mini Plasmid Kit Functional Test Data

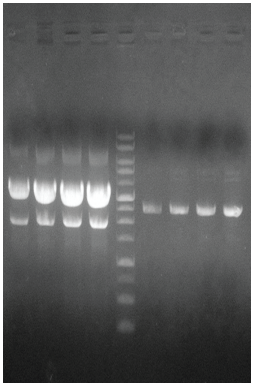

Figure 1. Plasmid DNA was extracted using the Presto™ Mini Plasmid Kit. 5 µl aliquots from a 100 µl eluate of purified super coiled plasmid DNA from 1.5, 3, 5 and 7 ml overnight *E. coli* (DH5 α) culture, containing a 3 kb plasmid pBluescript and pBR322 (OD600 = 4 U/ml) were used in *Eco*RI digestion and analyzed by electrophoresis on a 0.8% agarose gel.
M = Geneaid 1 Kb DNA Ladder
pBluescript: 1=1.5 ml, 2=3 ml, 3=5 ml, 4=7 ml
pBR322: 5=1.5 ml, 6=3 ml 7=5 ml, 8=7 ml

Copy Number	Host Strain Cell Culture Volume (OD600 = 4.0)			
	1.5 ml	3 ml	5 ml	7 ml
High-Copy (pBluescript)	13-15 µg	27-29 µg	36-38 µg	40-42 µg
Low-Copy (pBR322)	4-6 µg	8-10 µg	12-14 µg	18-20 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™ Midi Plasmid Kit	25 preps	PI025
Geneaid™ Midi Plasmid Kit (Endotoxin Free)	25 preps	PIE25
Presto™ Plasmid DNA Concentration Kit	250/500/1000 preps	PC0250/500/1000
Geneaid™ Maxi Plasmid Kit	10/25 preps	PM010/25
Geneaid™ Maxi Plasmid Kit (Endotoxin Free)	10/25 preps	PME10/25
Presto™ 96 Well Plasmid Kit	4/10 x 96 preps	96PDV04/10, 96PDC04/10