Presto™ 96 Well Plasmid Kit Quick Protocol

For research use only

Catalogue Number

96PDV02, 96PDV04, 96PDV10

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

IMPORTANT BEFORE USE!

1. Add provided RNase A to P1 Buffer then mix by shaking for a few seconds. Check the box on the bottle.
2. If precipitates have formed in P2 Buffer, warm 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.

Vacuum Protocol

1. Vacuum Manifold Preparation

Place the waste tray on the manifold base then place the binding top plate on the manifold base. Place the **Presto™ Plasmid 96 Well Binding Plate** in the binding top plate aperture. Place the filter top plate on the binding top plate. Place the **Presto™ Plasmid 96 Well Filter Plate** in the filter top plate aperture then attach the vacuum manifold to a vacuum source.

2. Resuspension

Add **200 µl of P1 Buffer (make sure RNase A was added)** to each well of the 96 Deep Well Plate or microcentrifuge tubes then resuspend the cell pellet by pipette. Continue to pipette until all traces of the cell pellet have been dissolved. Transfer the resuspended cell samples from microcentrifuge tubes to each well of a new 96 Deep Well Plate.

3. Lysis

Add **200 µl of P2 Buffer** to each well of the 96 Deep Well Plate. Dry the top of the plate with paper towel then seal tightly with new **Adhesive Film**. Gently invert the plate 6-8 times then incubate at room temperature for 2 minutes.

4. Neutralization

Briefly centrifuge the 96 Deep Well Plate at 2,000 x g to collect any sample mixture remaining on the **Adhesive Film**. Allow the centrifuge to reach 2,000 x g prior to stopping. Remove the **Adhesive Film** from the 96 Deep Well Plate then add **300 µl of P3 Buffer** to each well. Dry the top of the plate with paper towel then seal tightly with new **Adhesive Film**. Gently invert the plate 6-8 times.

5. Cell Debris Filtration

Briefly centrifuge the 96 Deep Well Plate at 2,000 x g to collect any sample mixture remaining on the **Adhesive Film**. Allow the centrifuge to reach 2,000 x g prior to stopping. Remove the **Adhesive Film** from the 96 Deep Well Plate. Transfer the lysate from each well (700 µl per well) to the wells of the **Presto™ Plasmid 96 Well Filter Plate (seal unused wells with adhesive film)**. Apply vacuum at 15 inches Hg until the lysate passes through completely (2-3 minutes) then switch off the vacuum.

6. DNA Binding

Remove the filter top plate and the **Presto™ Plasmid 96 Well Filter Plate**. Seal unused wells of the **Presto™ Plasmid 96 Well Binding Plate** with **Adhesive Film**. Apply vacuum at 15 inches Hg until the clear lysate passes through completely (approx.10 seconds) then turn off the vacuum.

7. Wash

Add **1 ml of Wash Buffer (make sure ethanol was added)** to each well of the **Presto™ Plasmid 96 Well Binding Plate**. Apply vacuum at 15 inches Hg until Wash Buffer passes through completely. Continue to apply vacuum for an additional 10 minutes to dry the membrane then turn off the vacuum.

8. Elution

Remove the **Presto™ Plasmid 96 Well Binding Plate** from the binding top plate aperture then blot the nozzles on a clean absorbent paper towel to remove residual ethanol. Remove the waste tray from the manifold base then place the collection plate spacer on the manifold base. Place a **0.35 ml collection plate** on top of the collection plate spacer. Place the top binding plate back on the manifold base then place the **96 Well Binding Plate** back in the binding top plate aperture. Add **100 µl of Elution Buffer**, TE or water into the **CENTER** of each well. Let stand for at least 2 minutes to ensure the Elution Buffer, TE or water is absorbed by the membrane. Apply vacuum at 15 inches Hg for 5 minutes then turn off the vacuum. Seal the 0.35 ml Collection Plate with Adhesive Film. Store the purified DNA at -20ºC. The average eluate volume is 60 µl from 100 µl elution buffer volume.

Centrifuge Protocol

1. Resuspension

Add **200 µl of P1 Buffer (make sure RNase A was added)** to each well of the 96 Deep Well Plate or microcentrifuge tubes then resuspend the cell pellet by pipette. Continue to pipette until all traces of the cell pellet have been dissolved. Transfer the resuspended cell samples from microcentrifuge tubes to each well of a new 96 Deep Well Plate.

2. Lysis

Add **200 µl of P2 Buffer** to each well of the 96 Deep Well Plate. Dry the top of the plate with paper towel then seal tightly with new **Adhesive Film**. Gently invert the plate 6-8 times then incubate at room temperature for 2 minutes.

3. Neutralization

Briefly centrifuge the 96 Deep Well Plate at 2,000 x g to collect any sample mixture remaining on the **Adhesive Film**. Allow the centrifuge to reach 2,000 x g prior to stopping. Remove the **Adhesive Film** from the 96 Deep Well Plate then add **300 µl of P3 Buffer** to each well. Dry the top of the plate with paper towel then seal tightly with new **Adhesive Film**. Gently invert the plate 6-8 times.

4. Cell Debris Filtration

Briefly centrifuge the 96 Deep Well Plate at 2,000 x g to collect any sample mixture remaining on the **Adhesive Film**. Allow the centrifuge to reach 2,000 x g prior to stopping. Place the **Presto™ Plasmid 96 Well Filter Plate** on a new 96 Deep Well Plate. Remove the Adhesive Film from the 96 Deep Well Plate containing the sample lysate. Transfer all sample lysate from each well (700 µl per well) to the wells of the **96 Well Filter Plate**. Centrifuge the **96 Well Filter Plate** and 96 Deep Well Plate together at 3,000 x g for 5 minutes then discard the **96 Well Filter Plate**.

5. DNA Binding

Place the **Presto™ Plasmid 96 Well Binding Plate** on top of a new 96 Deep Well Plate. Transfer the clear flow-through in each well of the 96 Deep Well Plate to each well of the **Presto™ Plasmid 96 Well Binding Plate**. Centrifuge the Binding Plate and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through then place the Binding Plate back on the 96 Deep Well Plate.

6. Wash

Add **500 µl of Wash Buffer (make sure ethanol was added)** to each well of the **Presto™ Plasmid 96 Well Binding Plate**. Centrifuge the **96 Well Binding Plate** and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Add another **500 µl of Wash Buffer** to each well. Centrifuge the **96 Well Binding Plate** and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through then place the **96 Well Binding Plate** back on the 96 Deep Well Plate. Centrifuge the **96 Well Binding Plate** and 96 Deep Well Plate together at 3,000 x g for 5 minutes to dry the membrane.

7. Elution

Remove the **Presto™ Plasmid 96 Well Binding Plate** from the 96 Deep Well Plate then blot the nozzles on a clean absorbent paper towel to remove residual ethanol. Place the **96 Well Binding Plate** on a **0.35 ml collection plate**. Add **100 µl of Elution Buffer**, TE or water into the **CENTER** of each well. Let stand for at least 2 minutes to ensure the Elution Buffer, TE or water is absorbed by the membrane. Centrifuge the **96 Well Binding Plate** and **0.35 ml collection plate** together at 3,000 x g for 5 minutes. Seal the **0.35 ml Collection Plate** with **Adhesive Film** and store the purified DNA at -20ºC. The average eluate volume is 60 µl from 100 µl elution buffer volume.

Components

Component	96PDV02	96PDV04	96PDV10
P1 Buffer[1]	40 ml	80 ml	200 ml
P2 Buffer[2]	40 ml	80 ml	200 ml
P3 Buffer	60 ml	120 ml	60 ml x 1 240 ml x 1
Wash Buffer[3] (Add Ethanol)	50 ml (200 ml)	50 ml x 2 (200 ml x 2)	50 ml x 4 (200 ml x 4)
Elution Buffer	30 ml	60 ml	120 ml
RNase A (50 mg/ml)	130 µl	260 µl	650 µl
Presto™ Plasmid 96 Well Filter Plate	2	4	10
Presto™ Plasmid 96 Well Binding Plate	2	4	10
0.35 ml Collection Plate	2	4	10
Adhesive Film	8	16	40

[1]Add provided RNase A to P1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. P1 Buffer and RNase A mixture should be stored at 2-8ºC for up to 6 months.

[2]If precipitates have formed in P2 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)

Troubleshooting

Low Yield

Incomplete buffer and cell culture preparation.

Add provided RNase A to P1 Buffer then mix by shaking for a few seconds. Check the box on the bottle then store at 2-8ºC for up to 6 months. If precipitates have formed in P2 Buffer, warm in a 37ºC water bath followed by gentle shaking to dissolve. 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. We recommend using a single freshly isolated *E. coli* colony to inoculate into 1.3 ml of LB medium. Solid and liquid medium should contain antibiotics. Do not use overgrown bacterial cultures (20-24 hours incubated in a 96 Deep Well Plate at 37ºC with 180-240 rpm shaking).

Culture growth medium was not removed completely.

Following centrifugation in the harvesting step, use a narrow pipette tip to ensure the supernatant is completely removed.

Cell pellet was not resuspended completely.

Resuspend the cell pellet completely by vortex or pipette. Continue to vortex or pipette until all traces of the cell pellet have been dissolved.

Bacterial cells were not lysed completely.

Using 2 OD600 - 4 OD600 units of bacterial culture is recommended. Following P2 Buffer addition, gently invert the plate 6-8 times then incubate at room temperature for 2 minutes.

Incorrect DNA Elution step.

Ensure that Elution Buffer, TE or water is added into the center of the well matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated Elution Buffer, TE, or water (60~70ºC). If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification.

Eluted DNA Does Not Perform Well In Downstream Applications

Residual ethanol/salt contamination.

Following the Wash Step, dry the binding plate with additional vacuum for 10 minutes at 15 inch Hg or centrifuge at 3,000-5,000 x g for 10 minutes.

RNA contamination.

Add provided RNase A to P1 Buffer then mix by shaking for a few seconds. Check the box on the bottle then store at 2-8ºC for up to 6 months.

Genomic DNA contamination.

Do not use overgrown bacterial cultures. Use only fresh cultures as they will contain less genomic DNA than old cultures. During P2 and P3 Buffer addition, mix gently to prevent genomic DNA shearing.

Nuclease contamination.

Following the DNA Binding step, add 400 µl of Endonuclease Removal Buffer (see page 11 of instruction manual) into each well of the binding plate. Apply vacuum until the buffer passes through completely or centrifuge at 3,000 x g for 5 minutes then proceed with Wash Buffer addition.