Presto™ 96 Well Plasmid Kit without Filter Plate Quick Protocol

For research use only

Catalogue Number

96PDC02, 96PDC04, 96PDC10

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.

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 (from 1-5 ml bacteria culture, OD_{600}=2-3) 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. DNA Binding

Centrifuge the 96 Deep Well Plate at 5,000 x g for 10 minutes. Following centrifugation, a cell debris pellet and clear lysate/supernatant will be present. Place the **Presto™ Plasmid 96 Well Binding Plate** on a new 96 Deep Well Plate. NOTE: There is a minimum height requirement of 75 mm for apparatus to hold the assembly of Binding Plate and Deep Well Collection Plate. Remove the **Adhesive Film** from the 96 Deep Well Plate then transfer the clear supernatant 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. Discard the flow-through then place the **96 Well Binding Plate** back on the 96 Deep Well Plate.

5. 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.

6. 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	96PDC02	96PDC04	96PDC10
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 Binding Plate	2	4	10
96 Deep Well Plate[4]	2	2	2
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.

[4]Two 96 Deep Well Plates are provided in each kit. After use, rinse the plates with water then incubate in 0.4M HCl for 1 minute at room temperature. Wash the plates thoroughly with ddH_2O. The plates can be autoclaved after being washed and re-used.

Storage

Dry at room temperature (15-25ºC)