Presto™ 96 Well Gel Extraction Kit Quick Protocol

For research use only

Catalogue Number

96DFG02, 96DFG04, 96DFG10

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 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.
2. It is important that the weight of the agarose gel slice does not exceed 300 mg. Please weigh each agarose gel slice before transferring to each well of the 96 Deep Well Plate.

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™ Gel Extraction 96 Well Binding Plate** in the binding top plate aperture. Seal unused wells of the **Presto™ Gel Extraction 96 Well Binding Plate** with **Adhesive Film** then attach the vacuum manifold to a vacuum source.

2. Gel Dissociation

Cut the agarose gel slice containing relevant DNA fragments and remove any extra agarose to minimize the size of the gel slice. Transfer **up to 300 mg of the gel slice** to each well of a 96 Deep Well Plate. Add **500 µl of QG Buffer** to each gel sample. Dry the top of the 96 Deep Well Plate with paper towel. Seal the plate tightly with **Adhesive Film** then mix by vortex. Incubate the plate at 55-60ºC in an oven or water bath for 10-15 minutes or until all gel slices are completely dissolved. During incubation, invert the plate every 3 minutes. If DNA fragments are larger than 5 kb, pre-heat the required Elution Buffer (80 µl per sample) to 60ºC (for Step 5 DNA Elution).

3. DNA Binding

Cool the sample mixture to room temperature then briefly centrifuge the 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 plate. If the color of the sample mixture has turned from yellow to purple, add **10 µl of 3M Sodium Acetate (pH5.0)** and mix thoroughly by pipetting. This will adjust pH of the sample mixture and the color will return to yellow. Transfer the sample mixture to each well of the **Presto™ Gel Extraction 96 Well Binding Plate**. Apply vacuum at 15 inches Hg until samples pass through completely (approximately 10 seconds) then turn off the vacuum.

4. Wash

Add **300 µl of W1 Buffer** to each well of the **Presto™ Gel Extraction 96 Well Binding Plate**. Apply vacuum at 15 inches Hg until **W1 Buffer** passes through completely (approximately 10 seconds) then turn off the vacuum. Add **600 µl of Wash Buffer (make sure ethanol was added)** to each well. Let stand for 1 minute then 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.

5. Elution

Remove the **Presto™ Gel Extraction 96 Well Binding Plate** from the binding top plate aperture and 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 binding top plate back on the manifold base then place the **Presto™ Gel Extraction 96 Well Binding Plate** back in the binding top plate aperture. Add **60-80 µl of Elution Buffer**, TE or water into the **CENTER** of each well of the **Presto™ Gel Extraction 96 Well Binding Plate**. Let stand for at least 3 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** and store the purified DNA at -20ºC. The average eluate volume is 60 µl from 80 µl elution buffer volume, and 40 µl from 60 µl elution buffer volume.

Centrifuge Protocol

1. Gel Dissociation

Cut the agarose gel slice containing relevant DNA fragments and remove any extra agarose to minimize the size of the gel slice. Transfer **up to 300 mg of the gel slice** to each well of a 96 Deep Well Plate. Add **500 µl of QG Buffer** to each gel sample. Dry the top of the 96 Deep Well Plate with paper towel. Seal the plate tightly with **Adhesive Film** then mix by vortex. Incubate the plate at 55-60ºC in an oven or water bath for 10-15 minutes or until all gel slices are completely dissolved. During incubation, invert the plate every 3 minutes. If DNA fragments are larger than 5 kb, pre-heat the required Elution Buffer (80 µl per sample) to 60ºC (for Step 5 DNA Elution).

2. DNA Binding

Cool the sample to room temperature and briefly centrifuge the plate at 2,000 x g to collect any mixture remaining on the adhesive film. Allow the centrifuge to reach 2,000 x g prior to stopping. Remove the **Adhesive Film** from the plate. If the color of the sample mixture has turned from yellow to purple, add **10 µl of 3M Sodium Acetate (pH5.0)** then mix thoroughly by pipetting. This will adjust pH of the sample mixture and the color will return to yellow. Place the **Presto™ Gel Extraction 96 Well Binding Plate** on a new 96 Deep Well Plate. Transfer the sample mixture to each well of the **Presto™ Gel Extraction 96 Well Binding Plate**. Centrifuge the **Presto™ Gel Extraction 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 **Presto™ Gel Extraction 96 Well Binding Plate** back on the 96 Deep Well Plate.

3. Wash

Add **300 µl of W1 Buffer** to each well of the **Presto™ Gel Extraction 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. Add **600 µl of Wash Buffer (make sure ethanol was added)** to each well. Let stand for 1 minute then 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 an additional 10 minutes to dry the membrane.

5. Elution

Remove the **Presto™ Gel Extraction 96 Well Binding Plate** from the 96 Deep Well Plate and 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 **60-80 µl of Elution Buffer**, TE or water to the **CENTER** of each well of the **96 Well Binding Plate**. Let stand for at least 3 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 80 µl elution buffer volume, and 40 µl from 60 µl elution buffer volume.

Components

Component	96DFG02	96DFG04	96DFG10
QG Buffer	125 ml	240 ml	275 ml x 2
3M Sodium Acetate (pH5.0)[1]	2 ml	2 ml	2 ml
W1 Buffer	80 ml	160 ml	200 ml x 2
Wash Buffer[2] (Add Ethanol)	25 ml (100 ml)	50 ml (200 ml)	25 ml + 50 ml x 2 (100 ml) (200 ml x 2)
Elution Buffer	30 ml	60 ml	100 ml
Presto™ Gel Extraction 96 Well Binding Plates	2	4	10
0.35 ml Collection Plates	2	4	10
Adhesive Film	6	12	30

[1]If the color of the mixture becomes purple instead of yellow once the gel slice is dissolved completely then the pH is too high. 3M Sodium Acetate (pH5.0) can then be added to adjust pH and the color will return to yellow.

[2]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

Agarose gel did not dissolve completely.

Ensure the agarose gel was melted/dissolved completely between 55-60ºC for 10-15 minutes, or until no gel is visible. If undissolved agarose remains in the sample, the 96 well plate could clog and some DNA will be unrecoverable. DNA can be denatured if the incubation temperature exceeds 60ºC. Do not use more than 300 mg of agarose gel per well.

Incomplete Wash Buffer preparation.

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.

Incorrect DNA elution step.

Ensure that Elution Buffer, TE or water is added into the **CENTER** of each well matrix and is completely absorbed. If DNA fragments are larger than 5 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

DNA was denatured (a smaller band appeared on gel analysis).

Ensure the agarose gel was melted/dissolved completely between 55-60ºC for 10-15 minutes, or until no gel is visible. DNA can be denatured if the incubation temperature exceeds 60ºC. Incubate the eluted DNA at 95ºC for 2 minutes then cool down slowly to reanneal the denatured DNA.