Large DNA Fragments Extraction Kit Quick Protocol

For research use only

Catalogue Number

DFL004, DFL100, DFL300

Instruction Manual Download

When using this product for the first time, or if you are unfammiliar with the procedure, please scan the QR code and download the complete instruction manual.

Instruction Manual Download

Gel Extraction Protocol

1. Gel Dissociation

Cut the TAE/TBE agarose gel slice containing relevant DNA fragments and remove any extra agarose to minimize the size of the gel slice. Transfer **up to 250 mg of the gel slice** to a 1.5 ml microcentrifuge tube. Add **600 µl of DF2 Buffer**. For >2% agarose gels, use 1.2 ml of DF2 Buffer.

2. DNA Binding

Resuspend **Presto™ Max Suspension** by vortex for 30 seconds. Add **10 µl of Presto™ Max Suspension** for <2 µg of DNA to the gel sample then mix the tube thoroughly by vortex.

NOTE: Add 20 µl of Presto™ Max Suspension for 2-5 µg of DNA

Incubate at 50ºC for 10-15 minutes to ensure the gel slice has dissolved completely. During incubation, vortex the tube every 2-3 minutes. If the color of the mixture has turned from yellow to purple, add 10 µl of Sodium Acetate (pH5.0) and mix thoroughly. This will adjust the pH and the color will return to yellow. Once the color has returned to yellow, incubate at room temperature for 5 minutes, mixing every 2 minutes. Centrifuge at 10,000 x g for 30 seconds to pellet the **Presto™ Max Suspension** then remove the supernatant using a pipette.

3. Wash

Add **500 µl of DF2 Buffer to the pelleted Presto™ Max Suspension**. Resuspend the pellet by brief vortex. Centrifuge at 10,000 x g for 30 seconds to pellet the **Presto™ Max Suspension**. Remove the supernatant with a pipette. Add **500 µl of Wash Buffer (make sure ethanol was added)** to the pelleted **Presto™ Max Suspension**. Resuspend the pellet by brief vortex. Centrifuge at 10,000 x g for 30 seconds to pellet the **Presto ™ Max Suspension**. Remove the supernatant with a pipette. Add **500 µl of Wash Buffer (make sure ethanol was added)** to the pelleted **Presto™ Max Suspension** again. Resuspend the pellet by brief vortex. Centrifuge at 10,000 x g for 30 seconds to pellet the **Presto™ Max Suspension**. Remove the supernatant with a pipette.

4. DNA Elution

Air-dry the **Presto™ Max Suspension** pellet at room temperature or 37ºC for 10-15 minutes with the cap open.

NOTE: Over drying the Presto™ Max Suspension will decrease DNA fragment recovery.

Add **10-20 µl of Elution Buffer**[1], TE[2] or water[3] then vortex to resuspend the **Presto™ Max Suspension**. Incubate the tube at 50ºC for 5 minutes. During incubation, vortex every 2 minutes.

NOTE: For larger DNA fragments (>10 kb), increase incubation time to 10-15 minutes.

Centrifuge for 1 minute at 10,000 x g to pellet the **Presto™ Max Suspension**. Carefully transfer the supernatant containing the purified DNA to a new 1.5 ml microcentrifuge tube.

NOTE: Do not touch the Presto™ Max Suspension pellet while transferring the supernatant.

NOTE: Repeating the elution step with an additional 10-20 µl of Elution Buffer will increase the yield by approximately 10-15%.

[1]Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC)

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications.

[3]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. DNA eluted in water should be stored at -20ºC to avoid degradation.

PCR Cleanup Protocol

1. Sample Preparation

Transfer up to **100 µl of reaction product** to a 1.5 ml microcentrifuge tube. Add **5 volumes of DF2 Buffer to 1 volume of the sample** and mix by vortex. If the color of the mixture has turned from yellow to purple, add **10 µl of Sodium Acetate (pH5.0)** and mix thoroughly. This will adjust the pH and the color will return to yellow.

2. DNA Binding

Resuspend **Presto™ Max Suspension** by vortex for 30 seconds. Add **10 µl of Presto™ Max Suspension per 5 µg of DNA** to the sample then mix the tube thoroughly by vortex. Incubate at room temperature for 10 minutes. During incubation, vortex the tube every 2-3 minutes. Centrifuge at 10,000 x g for 30 seconds to pellet the **Presto ™ Max Suspension** then remove the supernatant using a pipette.

3. Wash

Add **500 µl of Wash Buffer (make sure ethanol was added)** to the pelleted **Presto™ Max Suspension**. Resuspend the pellet by brief vortex. Centrifuge at 10,000 x g for 30 seconds to pellet the **Presto™ Max Suspension.** Remove the supernatant with a pipette. Add **500 µl of Wash Buffer (make sure ethanol was added)** to the pelleted **Presto™ Max Suspension** again. Resuspend the pellet by brief vortex. Centrifuge at 10,000 x g for 30 seconds to pellet the **Presto™ Max Suspension** then remove the supernatant with a pipette.

4. DNA Elution

Air-dry the **Presto™ Max Suspension** pellet at room temperature or 37ºC for 10-15 minutes with the cap open.

NOTE: Over drying the Presto™ Max Suspension will decrease DNA fragment recovery.

Add **10-20 µl of Elution Buffer**[1], TE[2] or water[3] then vortex to resuspend the **Presto™ Max Suspension**. Incubate the tube at 50ºC for 5 minutes. During incubation, vortex every 2 minutes.

NOTE: For larger DNA fragments (>10 kb), increase incubation time to 10-15 minutes.

Centrifuge for 1 minute at 10,000 x g to pellet the **Presto™ Max Suspension**. Carefully transfer the supernatant containing the purified DNA to a new 1.5 ml microcentrifuge tube.

NOTE: Do not touch the Presto™ Max Suspension pellet while transferring the supernatant.

NOTE: Repeating the elution step with an additional 10-20 µl of Elution Buffer will increase the yield by approximately 10-15%.

[1]Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC)

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications.

[3]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. DNA eluted in water should be stored at -20ºC to avoid degradation.

Kit Components

Component	DFL004	DFL100	DFL300
Presto™ Max Suspension	60 µl	0.75 ml x 2	0.75 ml x 6
DF2 Buffer[1]	4.5 ml	120 ml	240 ml x 1 120 ml x 1
3M Sodium Acetate (pH5.0)[2]	N/A	200 µl	200 µl
Wash Buffer[3] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml + 25 ml (200 ml) (100 ml)
Elution Buffer	1 ml	6 ml	30 ml

[1]Routine purification from >2% agarose gel requires additional DF2 Buffer.

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

[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)