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IBI SCIENTIFIC

INSTRUCTION MANUAL

Large DNA Fragments Extraction Kit

IB47070 (4 Prep Sample Kit)

IB47071 (100 Prep Kit)

IB47072 (300 Prep Kit)

Advantages

Ecient: purify large DNA fragments within 20 minutes
Sample: up to 250 mg of either TAE or TBE agarose gel, up to 100 µl of PCR products
Yield: up to 50 µg of pure plasmid DNA
Fragment Size: 100 bp-50 kb
Recovery: 100 bp - 10 kb = 70-95%, >10 kb = 60-80%
Format: modified silica bead protocol
Convenient: includes pH indicator for easy determination of optimal pH and sodium acetate to adjust pH if it becomes too high following gel dissociation or PCR product reaction
Binding Capacity: 5 µg of DNA per 10 µl of Silica Beads
Operation Time: 20 minutes for PCR Cleanup and 30 minutes for Gel Extraction
Elution Volume: 10-20 µl
Kit Storage: dry at room temperature (15-25°C)

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Introduction

The Large DNA Fragments Extraction Kit was designed to recover or concentrate a broad range of DNA fragments (100 bp - 50 kb) from agarose gel, PCR, or other enzymatic reactions. A concentrated sodium buffer is used to dissolve agarose gel and denature enzymes. DNA fragments are then bound by silica beads. Contaminants are removed with Wash Buffer (containing ethanol) and the purified DNA fragments are eluted by a low salt Elution Buffer, TE Buffer or water. Salts, enzymes and unincorporated nucleotides can be effectively removed from the reaction mixture without phenol extraction or alcohol precipitation. 10-20 µl of Elution Buffer is used to obtain a high concentration of purified DNA which is ready for use in downstream applications.

Quality Control

The quality of the Large DNA Fragments Extraction Kit is tested on a lot-to-lot basis by purifying DNA fragments from either agarose gel, PCR products or other aqueous solutions and analyzed by electrophoresis.

Kit Components

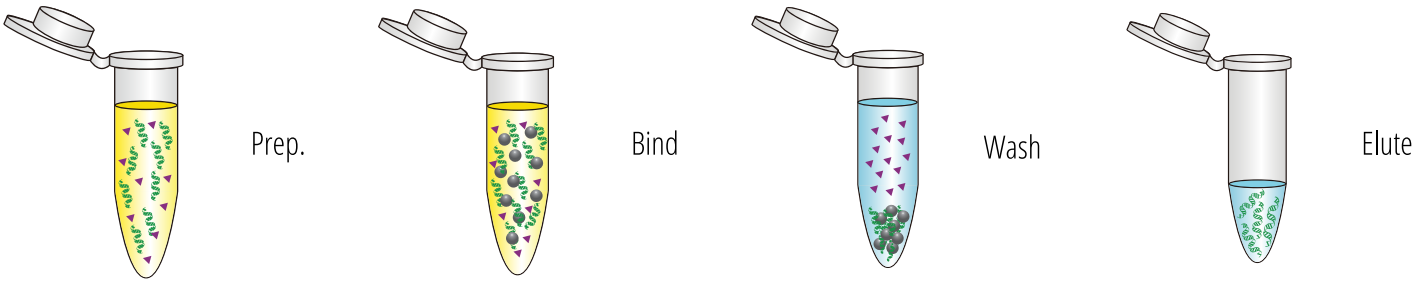
Component	IB47070	IB47071	IB47072
Silica Beads Suspension	60 µl	0.75 ml x 2	0.75 ml x 6
DF2 Buffer[1]	4.5 ml	120 ml	240 ml x 1 120 ml x 1
3M Sodium Acetate (pH5.0)[2]	N/A	200 µl	200 µl
Wash Buffer[3] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml + 25 ml (200 ml) (100 ml)
Elution Buffer	1 ml	6 ml	30 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.

Quick Protocol Diagram



Large DNA Fragments Extraction Kit Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.
During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

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. Record the weight of an empty 1.5 ml microcentrifuge tube for the Gel Dissociation step. Once the gel has been transferred to the tube, record the weight again. Subtract the empty tube weight from the total weight to determine the actual gel weight.
3. Set heating block or water bath at 50°C
4. It is not necessary to remove mineral oil or kerosene from the PCR sample prior to cleanup.

Additional Requiremens:
1.5 ml microcentrifuge tubes, absolute ethanol

Protocol Procedure With Color Indicator

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.**

NOTE! For >2% agarose gels, use 1.2 ml of DF2 Buffer.

2. DNA Binding

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

NOTE! Add 20 µl of Silica Beads Suspension for 2-5 µg of DNA.

Incubate at 50°C 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 thorouhgly. 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 mintues, mixing every 2 minutes. Centrifuge at 10,000 x g for 30 seconds to pellet the **Silica Beads Suspension** then remove the supernatant using a pipette.

pH Indicator

Optimal pH

pH Too High

A pH indicator is premixed with the DF2 Buffer to ensure optimal pH, facilitate DNA binding and allow for easy observation of undissolved agarose gel. If pH exceeds the optimal level (>7.5), the color of the solution will appear purple instead of yellow. 3M Sodium Acetate (pH5.0), which is included with the kit, can then be added to the solution to adjust pH and return the color to yellow.

3. Wash

Add 500 µl of DF2 Buffer to the pelleted Silica Beads Suspension then resuspend the pellet by brief vortex. Centrifuge at 10,000 x g for 30 seconds to pellet the Silica Beads Suspension then remove the supernatant with a pipette. Add 500 µl of Wash Buffer (make sure ethanol was added) to the pelleted Silica Beads Suspension then resuspend the pellet by brief vortex. Centrifuge at 10,000 x g for 30 seconds to pellet the Silica Beads Suspension. Remove the supernatant with a pipette. Repeat the wash step with an additional 500 µl ofWash Buffer (make sure ethanol was added). Centrifuge at 10,000 x g for 30 seconds to pellet the Silica Beads Suspension t en remove the supernatant with a pipette.

4. Elution

Air-dry the **Silica Beads Suspension** pellet at room temperature or 37°C for 10-15 minutes with the cap open.

NOTE! NOTE: Over drying the Silica Beads Suspension will decrease DNA fragment recovery. Add 1**0-20 µl of Elution Buffer**[1], TE[2] or water[3] then vortex to resuspend the **Silica Beads Suspension**. Incubate the tube at 50°C for 5 minutes. During incubation, vortex every 2 minutes.

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 **Silica Beads Suspension**. Carefully transfer the supernatant containing the purified DNA to a new 1.5 ml microcentrifuge tube. Do not touch the Silica Beads Suspension pellet while transferring the supernatant.

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 25°C)

[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 -20°C to avoid degradation.

PCR Cleanup Protocol Procedure

1. PCR Cleanup 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 thorouhgly. This will adjust the pH and the color will return to yellow.

2. DNA Binding

Resuspend **Silica Beads Suspension** by vortex for 30 seconds. Add **10 µl of Silica Beads 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 **Silica Beads Suspension** then remove the supernatant using a pipette.

3. Wash

Add **500 µl of Wash Buffer (make sure ethanol was added)** to the pelleted **Silica Beads Suspension** then resuspend the pellet by brief vortex. Centrifuge at 10,000 x g for 30 seconds to pellet the **Silica Beads Suspension** then remove the supernatant with a pipette. Repeat the wash step with an additional **500 µl of Wash Buffer (make sure ethanol was added)**. Centrifuge at 10,000 x g for 30 seconds to pellet the **Silica Beads Suspsion** then remove the supernatant with a pipette.

4. Elution

Air-dry the **Silica Beads Suspension** pellet at room temperature or 37°C for 10-15 minutes with the cap open.

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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 Silica Beads Suspension. Carefully transfer the supernatant containing the purified DNA to a new 1.5 ml microcentrifuge tube. Do not touch the **Silica Beads Suspension** pellet while transferring the supernatant.

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

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[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 -20°C to avoid degradation.

Troubleshooting

Low Yield

Agarose gel did not dissolve completely.

Ensure the agarose gel was melted/dissolved completely between 50-60°C for 10-15 minutes, or until no gel is visible. If undissolved agarose remains in the sample, some DNA will be unrecoverable. DNA can be denatured if the incubation temperature exceeds 60°C. If using more than 250 mg of agarose gel, separate it into multiple 1.5 ml microcentrifuge tubes. Mix the tube every 2 minutes during incubation to ensure the silica matrix remains in suspension

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.

If using water for elution, ensure the water pH is ≥8.0. dd H_2O should be fresh as ambient CO_2 can quickly cause acidification. Repeating the elution step with an additional 10-20 µl of Elution Buffer will increase the yield by approximately 10-15%.

pH is Too High (pH>7.5, the color of the mixture has turned from yellow to purple)

Add 10 µl of 3M Sodium Acetate (pH5.0) to the sample mixture then mix thoroughly. This will adjust pH and the color of the sample will return to yellow indicating the appropriate pH to facilitate DNA binding.

Over-dried Silica Beads Suspension Pellet.

Do not dry the Silica Beads Suspension pellet by vacuum centrifuge.

Eluted DNA Does Not Perform Well In Downstream Applications

Incomplete Removal of DF2 Buffer.

Salt from DF2 Buffer may inhibit subsequent enzymatic reactions. Wash the Silica Beads Suspension pellet twice with Wash Buffer.

Residual Ethanol Contamination.

Following the wash step, dry the Silica Beads Suspension pellet at room temperature or 37°C for 10-15 minutes with the cap open. Residual ethanol from the Wash Buffer will inhibit downstream applications and must be removed completely.

Large DNA Fragments Extraction Kit Functional Test Data

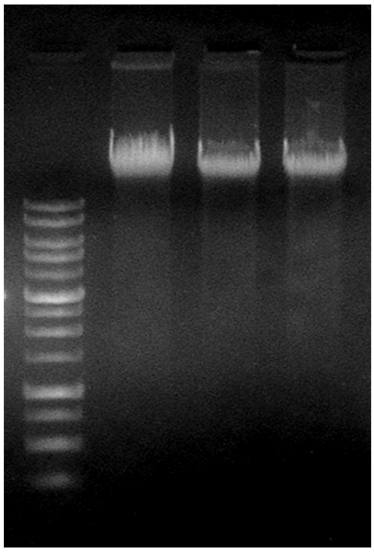

Figure 1. Genomic DNA was extracted from 1x10^6 Hela cells using a traditional phenol-chloroform method combined with ethanol precipitation. 2 µg of extracted genomic DNA (30 kb) was then purified using the Large DNA Fragments Extraction Kit. The quality and concentration of genomic DNA was significantly increased following the purification process. M = 1 Kb DNA Ladder.

Copy Number	ng/µl	260/280	260/230	Yield (µg)	Recovery
1 – Unpurified 30kb gDNA	20	1.67	0.92	2.0	---------
2 – Purified 30kb gDNA	64.8	1.89	1.74	1.3	65%
3 – Purified 30kb gDNA	61.5	1.87	1.75	1.23	61.5%

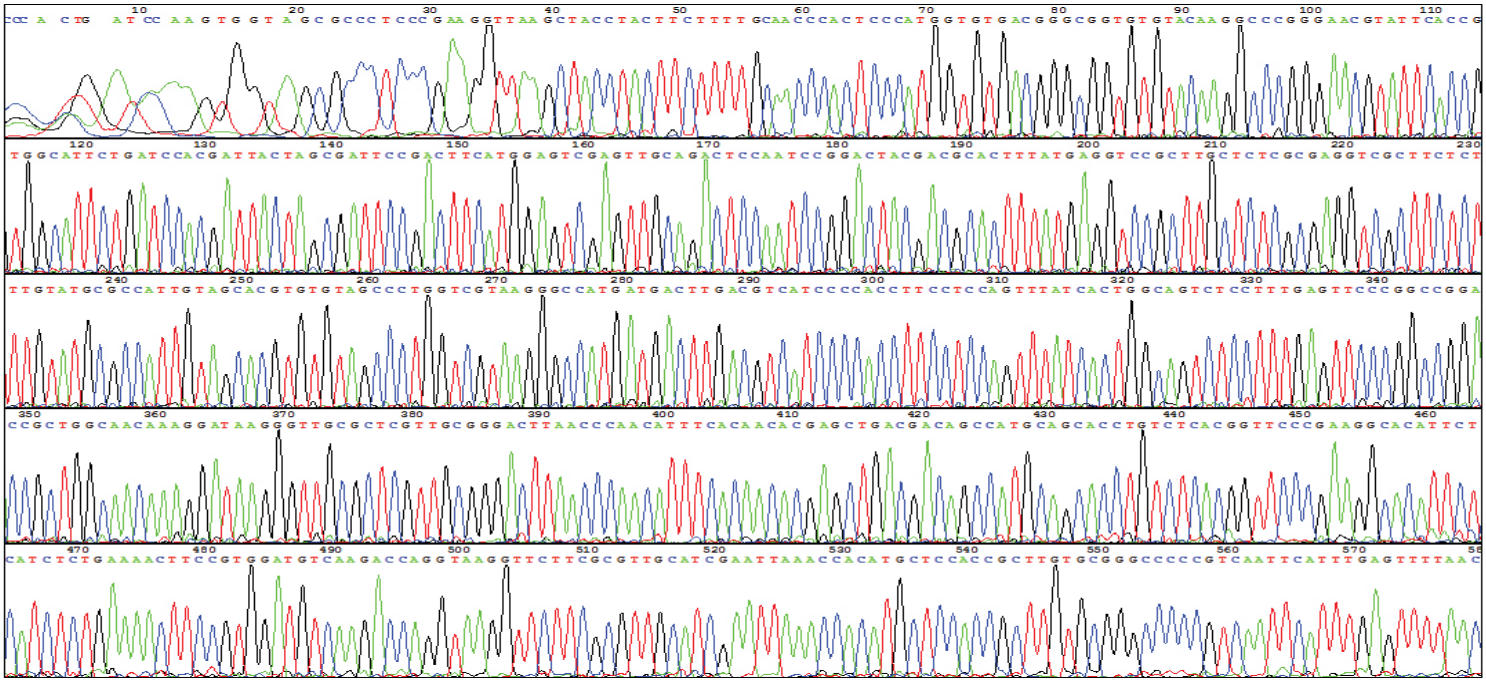

Figure 2. Sequencing data of DNA purified using the Large DNA Fragments Extraction Kit.

Related DNA Extraction Products

Post Reaction DNA Purification		
Product	Package Size	Catalogue Number
Gel/PCR DNA Fragments Extraction Kit	100/300 preps	IB47020/030
Small DNA Fragments Extraction Kit	100/300 preps	IB47061/062
Large DNA Fragments Extraction Kit	100/300 preps	IB47071/072

For additional product information please visit **www.ibisci.com**. Thank you!

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