

UltraClean™ GelSpin™ DNA Purification Kit

Catalog # 12400-50
50 preps

Instruction Manual

UltraClean™ GelSpin Agarose Gel DNA Purification Kit

Introduction

Use this spin filter format kit for isolating DNA from agarose gels and cleaning up or purifying DNA from reactions or solutions. You can isolate DNA from all types of agarose gels and buffer systems in 5 minutes. It is not necessary to use low melt agarose to get high yields. This kit will remove salts, buffers, enzymes, ethidium bromide, label, and dNTPs from reactions. The resulting DNA can be used for any downstream application.

Precautions

Please wear gloves when using this product. Avoid all skin contact with reagents in this kit. In case of contact wash thoroughly with water. Do not ingest. See Material Safety Data Sheets for emergency procedures in case of accidental ingestion or contact. All MSDS information is available upon request (760-929-9911) or on our web site at www.mobio.com. Reagents labeled flammable should be kept away from open flames and fire.

This kit is for research purposes only. Not for diagnostic use.

Equipment required:

Microcentrifuge (13,000 x g)

Kit Contents: 50 purifications

<u>Component</u>	<u>Amt.</u>	<u>Description</u>
GelBind Buffer	33 ml	NaClO ₄ solution
GelWash	16.5 ml	Tris/ Ethanol solution
Elution Buffer	3.3 ml	10 mM Tris pH 8.0
Spin Filter units	50	Spin filter in centrifuge tube
Collection tubes	50	2.0 ml centrifuge tubes

Specifications:

DNA Size range: 100 bp - 50 kb
Types of agarose: All
Types of gel buffers: All
Spin Filter binding capacity: 20 µg
Final volume of DNA: 50 µl
Recovery rates: 80-100%

Kit Storage

Room temperature for 1 year.

Detailed Protocol: (Explains what is occurring at each step)

To isolate DNA from an Agarose gel

1. Cut desired DNA band from a TAE or TBE agarose gel.
What's happening: The agarose gel slice containing the desired DNA is removed from the rest of the gel.
2. Determine gel band weight. This can be done in a spin filter or separate tube.
3. The following procedures should be carried out directly in the spin filter (for up to 0.2 gm of gel).
For > 0.2 gm or to process in a separate tube, see Hints page for procedure.
4. Place gel in the spin filter basket. Be sure gel is resting on the white filter membrane. The maximum capacity per spin filter is 0.2 gm.
What's happening: By placing the gel directly in the spin filter you can save tubes and time. The gel will be melted in the spin filter.
5. Add 3 volumes of GelBind to the gel slice. For example, 0.1 gm agarose requires 0.3 ml (300µl) of GelBind. Be sure gel is submerged in the GelBind buffer. Close lid.
6. Incubate for 2 minutes at 55°C. Invert once. Incubate one minute more or until gel is melted. (> 2% gels can require more than 5 minutes to melt).
What's happening: The gel should melt within a few minutes or longer depending on the agarose percentage. Gels above 1.2% can take up to 5 minutes to melt. At this point the gel should be irreversibly melted. It will not re-solidify at room temperature.
7. Invert once to mix.
What's happening: The mixing step is important to keep the proper salt concentration.
8. Centrifuge spin filter 10 seconds at 10,000 x g.
What's happening: The melted gel and unwanted salts flow through the spin filter membrane as the DNA binds to the membrane.
9. Very important. Remove the spin filter. Vortex the collection tube for 5 seconds to mix the flow through.
What's happening: When the gel melted, the DNA entered a salt solution. The gel however, does not melt evenly. In most cases all the DNA does not bind on the first pass through the spin filter. The mixing step makes the salt concentration homogeneous.
10. Reload all the liquid from collection tube back onto the spin filter.
What's happening: Passing the DNA through the spin filter a second time will now allow any unbound DNA to bind to the spin filter. This greatly increases the yield of DNA from the melted agarose.
11. Centrifuge 10 seconds at 10,000 x g.
12. Discard flow through liquid and replace spin filter basket.
What's happening: All available DNA should be bound to the spin filter membrane. The discarded liquid contains only melted agarose and salt.
13. Add 300 µl GelWash buffer.
What's happening: GelWash is composed of an ethanol solution. It keeps the DNA bound to the spin filter while washing away residual salt and melted agarose.
14. Centrifuge 10 seconds at 10,000 x g.
15. Discard flow through and centrifuge again for 30 seconds at 10,000 x g.
What's happening: It is important to dry out any residual GelWash because the ethanol contained in this buffer can inhibit down stream applications for the recovered DNA.
16. Carefully transfer filter basket to a clean collection tube provided.
17. Add 50µl of Elution buffer (10 mM Tris) or water directly onto the center of the white spin filter membrane.
What's happening: The elution buffer must contact the entire surface of the spin filter membrane for efficient recovery of the DNA. The DNA is released from the spin filter membrane at this point because there is no salt to keep it bound.
18. Centrifuge 30 seconds at 10,000 x g. Discard filter basket.
What's happening: The DNA flows through the spin filter membrane and into the collection tube.
19. DNA is now ready to use. Thank you for choosing the UltraClean GelSpin Kit.

To clean up DNA from a reaction solution.

Introduction

This process will clean up or purify DNA. It removes salts from reaction buffers, enzymes, label, dNTPs, ethidium bromide, and all other unwanted reaction components.

1. Add 3 volumes of GelBind to the DNA solution. For example, 0.1 ml (100 μ l) reaction requires 0.3 ml (300 μ l) of GelBind.
2. Mix well by Pipetting up and down.
What's happening: The GelBind creates a high salt condition that is required for binding DNA.
3. Add mixture to a new spin filter unit.
What's happening: The mixing step is important to make a homogeneous salt concentration. The DNA/salt mixture will bind to the white spin filter membrane inside the spin filter unit.
4. Close spin filter tube lid and centrifuge for 10 seconds at 10,000 x g.
What's happening: Unwanted salts, buffers, enzymes and other components flow through the spin filter membrane as the DNA binds to the membrane.
5. Discard flow through liquid and replace spin filter basket in the same tube.
What's happening: All available DNA should be bound to the spin filter membrane in the spin filter basket. The discarded liquid contains only unwanted components.
6. Add 300 μ l GelWash buffer.
What's happening: GelWash is composed of an ethanol solution. It keeps the DNA bound to the spin filter while washing away residual contaminants.
7. Centrifuge 10 seconds at 10,000 x g.
8. Discard flow through and centrifuge again for 30 seconds at 10,000 x g.
What's happening: It is important to dry out any residual GelWash because the ethanol contained in this buffer can inhibit down stream applications for the recovered DNA.
9. Carefully transfer filter basket to a clean collection tube provided.
10. Add 50 μ l of Elution buffer (10 mM Tris) or water directly onto the center of the white spin filter membrane.
What's happening: The elution buffer must contact the entire surface of the spin filter membrane for efficient recovery of the DNA. The DNA is released from the spin filter membrane at this point because there is no salt to keep it bound.
11. Centrifuge 30 seconds at 10,000 x g. Discard filter basket.
What's happening: The DNA flows through the spin filter membrane and into the collection tube with the elution buffer.
12. DNA is now ready to use. Thank you for choosing the UltraClean GelSpin Kit.

Short Protocols:

Please wear gloves

All of the centrifugation steps are 10,000 x g.

To isolate DNA from an Agarose gel

1. Cut desired DNA band from a TAE or TBE agarose gel.
2. Determine gel band weight. The following procedures should be carried out directly in the spin filter (for up to 0.2 gm of gel). For > 0.2 gm or to process in a separate tube see Hints page for procedure.
3. Place gel in the spin filter basket. Be sure gel is resting on the filter. The maximum capacity per spin filter is 0.2 gm.
4. Add 3 volumes of GelBind to the gel slice. For example, 0.1 gm agarose requires 0.3 ml (300 μ l) of GelBind. Be sure gel is submerged in the GelBind buffer. Close lid.
5. Incubate for 2 minutes at 55°C. Invert once. Incubate one minute more or until gel is melted. (> 2% gels can require more than 5 minutes to melt).
6. Invert once to mix.
7. Centrifuge spin filter 10 seconds at 10,000 x g.
8. Very important. Remove the spin filter. Vortex the collection tube for 5 seconds to mix the flow through.
9. Reload all the liquid from collection tube back onto the spin filter.
10. Centrifuge 10 seconds at 10,000 x g.
11. Discard flow through liquid and replace spin filter basket.
12. Add 300 μ l GelWash buffer.
13. Spin 10 seconds.
14. Discard flow through and spin again for 30 seconds.
15. Carefully transfer filter basket to a clean collection tube provided.
16. Add 50 μ l of Elution buffer (10 mM Tris) or water directly onto the center of the white spin filter membrane.
17. Centrifuge 30 seconds at 10,000 x g. Discard filter basket.
18. DNA is now ready to use. Thank you for choosing the UltraClean GelSpin Kit.

To clean up DNA from a reaction solution

1. Add 3 volumes of GelBind to the DNA solution. For example, 0.1 ml (100 μ l) reaction requires 0.3 ml (300 μ l) of GelBind.
2. Mix by Pipetting up and down.
3. Centrifuge spin filter 10 seconds at 10,000 x g.
4. Discard flow through liquid and replace spin filter basket.
5. Add 300 μ l GelWash buffer.
6. Spin 10 seconds.
7. Discard flow through and spin again for 30 seconds.
8. Carefully transfer filter basket to a clean collection tube provided.
9. Add 50 μ l of Elution buffer (10 mM Tris) or water directly onto the center of the white spin filter membrane.
10. Centrifuge 30 seconds at 10,000 x g. Discard filter basket.
11. DNA is now ready to use.

Thank you for choosing the UltraClean GelSpin Kit.

Hints and Troubleshooting Guide

Concentrating the DNA

Your final volume will be 50µl. If this is too dilute for your purposes, add 2 µl of 5M NaCl and mix. Then add 100 µl of 100% cold ethanol. Mix. Centrifuge at 10,000 x g for 5 minutes. Decant all liquid. Dry residual ethanol in a speed vac or dessicator or ambient air. Resuspend precipitated DNA in desired volume.

Melting the gel slice

Make sure you add GelBind before attempting to melt the gel.. Always make sure you have melted the gel completely at 55°C before proceeding to step 6. If tube is floating too high out of water, the gel will take longer to melt. Inversion in steps 5 and 6 are critical to facilitate melting.

Low recovery (below 50% recovery is considered poor)

1. Gel was not completely melted before proceeding to step 7.
2. Melting temperature was too high.
3. Centrifuge does not spin with sufficient force.
4. Elution buffer was not loaded directly onto center of spin filter.

For gel slices over 0.2 grams in weight.

Place gel band in 2 ml tube. Do steps 2,4,5. of protocol. Add up to 700 µl from step 5 to a spin filter. Spin 10 seconds. Reload flow through from collection tube back into spin filter and spin 10 seconds. Discard flow through. Add remaining melted gel to spin filter. Spin 10 seconds. Reload flow through. Spin 10 seconds. Go to step 10 and continue.

Other UltraClean™ Kits available from Mo Bio Laboratories, Inc.

<u>Kit description</u>	<u>Cat. number</u>
Plasmid Prep Kits	
6 minute Mini Plasmid Prep Kit (100 preps)	12300-100
6 minute Mini Plasmid Prep Kit (250 preps)	12300-250
25-50 ml Plasmid Prep Kit (20 preps)	12700-20
25-50 ml Plasmid Prep Kit (50 preps)	12700-50
250-500 ml Plasmid Prep Kit (10 preps)	12600-10
250-500 ml Plasmid Prep Kit (20 preps)	12600-20
Endotoxin-Free Plasmid Prep Kits	
Endotoxin-free Mini Prep Kit (100 preps)	12311-100
Endotoxin-free Mini Prep Kit (250 preps)	12311-250
Endotoxin-free Midi Prep Kit (10 preps)	12711-10
Endotoxin-free Maxi Prep Kit (10 preps)	12611-10
DNA Purification Kits	
Agarose Gel DNA Purification Kit (300 preps)	12100-300
Agarose Gel-Spin DNA Purification (100 preps)	12400-100
Agarose Gel-Spin DNA Purification (250 preps)	12400-250
PCR Clean-Up Kit (100 preps)	12500-100
PCR Clean-Up Kit (250 preps)	12500-250
DNA Isolation Kits	
DNA Blood Isolation Kit (100 preps)	12000-100
DNA BloodSpin Kit (50 preps)	12200-50
DNA BloodSpin Kit (250 preps)	12200-250
Mega BloodSpin Kit (10 preps)	12210-10
Soil DNA Isolation Kit (50 preps)	12800-50
Soil DNA Isolation Kit (100 preps)	12800-100
Soil DNA Mega Prep Kit (10 preps)	12900-10
Fecal DNA Isolation Kit (50 preps)	12811-50
Fecal DNA Isolation Kit (100 preps)	12811-100
Microbial DNA Isolation Kit (50 preps)	12224-50
Microbial DNA Isolation Kit (250 preps)	12224-250
Plant DNA Isolation Kit (50 preps)	13000-50
Plant DNA Isolation Kit (250 preps)	13000-250
Tissue DNA Isolation Kit (50 preps)	12334-50
Tissue DNA Isolation Kit (250 preps)	12334-250
Water DNA Isolation Kit (10 preps)	14800-10
Water DNA Isolation Kit (25 preps)	14800-25
Forensic DNA Kit- Single prep format (10 preps)	14000-10
Forensic DNA Kit- Single prep format (20 preps)	14000-20
RNA Isolation Kits	
Tissue RNA Isolation Kit (50 preps)	15000-50
Tissue RNA Isolation Kit (250 preps)	15000-250
Plant RNA Isolation Kit (20 preps)	13300-20
Plant RNA Isolation Kit (50 preps)	13300-50
Microbial RNA Isolation Kit (50 preps)	15800-50
Microbial RNA Isolation Kit (250 preps)	15800-250
Growth Media	
TB DRY (1 kg) Terrific Broth powder	12105-1
LB (1 kg) LB powder (Miller)	12106-1
LB Agar (1 kg) LB Agar Powder (Miller)	12107-1



Technical information:

Call Mo Bio Laboratories, Inc. Toll free 1-800-606-6246, or 1-760-929-9911 email technical@mobio.com
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Ordering Information

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For the distributor nearest you, go to our web site at www.mobio.com/distributors/