



UltraClean™ BloodSpin™ Kit

Catalog # 12200-50
50 preps

Instruction Manual

Introduction

Use this kit for isolating Genomic DNA from 200µl of whole blood. This spin filter method kit yields PCR ready DNA.

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.

Avoid contact with bleach or other oxidizers.

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

Equipment required:

Microcentrifuge (13,000 x g)

65°C water bath

Pipettor (volumes required 20 µl - 500 µl), vortex

Kit Contents: Sufficient reagents for 50 preps of 200µl.

Note: an economical 250 prep kit is available. Cat# 12200- 250

<u>Component</u>	<u>Amount</u>	<u>Description</u>
Proteinase K*	6.25mg	Proteinase K*

***Before starting protocol, add 1.2 ml of distilled water to lyophilized Proteinase K**

Solution B1	11 ml	Guanidine HCl/Tween solution
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Solution B2	11 ml	100% Ethanol
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Solution B3	27.5 ml	Ethanol/Guanidine HCl solution
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Solution B4	30 ml	Ethanol, Tris, NaCl.
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Solution B5	11 ml	10 mM Tris-HCl.
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Spin Filters	50	Spin filter basket in a 2.0 ml tube.
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2.0 ml tubes	150	Microcentrifuge tubes.
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Kit Storage

Remove Proteinase K and store at -20°C. All other components room temperature. Proteinase K is stable for 2 months after reconstituting at 4°C. To prolong life, aliquot out into smaller amounts, and store at -20°C.

WARNING: Solution B2, B3, and B4 are flammable.



Protocol

Please wear gloves at all times

1. See "Kit Contents" before starting to prepare Proteinase K.
2. Add 200 μ l of whole blood to 20 μ l of Proteinase K.
3. (optional: RNase A can be added at this step. Use 2 μ l of 20mg/ml.)
4. Add 200 μ l of Solution B1.
5. Bump vortex for 15 seconds.
6. Incubate at 65°C for 10 min.
7. Centrifuge briefly to remove any drops from the top of the lid.
8. Add 200 μ l of Solution B2 and vortex for 15 sec.
9. Centrifuge briefly to remove any drops from the top of the lid.
10. Transfer all of the mixture to the spin filter.
11. Centrifuge 1 min at 13,000xg.
12. Transfer spin filter to new tube.
13. Add 500 μ l of Solution B3 to spin filter.
14. Centrifuge 30 seconds at 13,000xg.
15. Remove spin filter, dump flow through, and replace spin filter.
16. Add 500 μ l of Solution B4 to spin filter.
17. Centrifuge 30 seconds at 13,000xg.
18. Remove spin filter, dump flow through, and replace spin filter.
19. Centrifuge again for 30 seconds at 13,000xg.
20. Carefully remove spin filter and transfer to a new tube without it coming in contact with the wash.
21. Add 200 μ l of Solution B5. To increase yields, incubate for 5 min. at 65°C.
22. Centrifuge 1 min. at 13,000xg.
23. Remove filter unit and close tube lid. Genomic DNA in tube is now ready to use for any application.

Thank you for choosing the UltraClean™ BloodSpin Kit.

To concentrate DNA, if desired, see Hints on next page.

Detailed Protocol (Describes each step)

Please wear gloves at all times

1. See “Kit Contents” before starting to prepare Proteinase K.

What’s happening: The Proteinase K solution requires reconstituting the powdered Proteinase K into distilled water before using it the first time.

2. Add 200µl of whole blood to 20µl of Proteinase K.

What’s happening: Proteinase K breaks down the cell wall and helps lyse the cell.

3. (optional: RNase A can be added at this step. Use 2µl of 20mg/ml.)

What’s happening: This kit selectively removes most RNA. However, to insure complete removal, add RNase A at this step. No incubation is necessary.

4. Add 200µl of Solution B1.

What’s happening: Solution B1 is a lysis reagent. It contains a chaotropic salt and detergent. Solution B1 breaks open the cells.

5. Bump vortex for 15 seconds.

What’s happening. Bump vortexing is a gentle mixing method. Here the tube is placed on and off the vortex over a period of 15 seconds.

6. Incubate at 65°C for 10 minutes.

What’s happening: Heat helps denature proteins.

7. Centrifuge briefly to remove any drops from the top of the lid.

8. Add 200µl of Solution B2 and vortex for 15 seconds.

What’s happening: Solution B2 precipitates denatured proteins.

9. Centrifuge briefly to remove any drops from the top of the lid.

10. Transfer all of the mixture to the spin filter.

11. Centrifuge 1 minute at 13,000 x g.

What’s happening: DNA binds to the silica membrane in the spin filter because it is in a high salt condition. The liquid flow through contains unwanted cell material such as denatured proteins and RNA.

12. Transfer spin filter to new tube.

13. Add 500µl of Solution B3 to spin filter.

What’s happening: Solution B3 is a salt based wash solution that cleans the DNA bound to the spin filter.

14. Centrifuge 30 seconds at 13,000 x g.

15. Remove spin filter, discard flow through, and replace spin filter.

What’s happening: The flow through contains residues of unwanted cell debris.

16. Add 500µl of Solution B4 to spin filter.

What’s happening: Solution B4 is an ethanol based wash solution that cleans the DNA bound to the spin filter. It removes all residual salt contaminants.

17. Centrifuge 30 seconds at 13,000xg.

18. Remove spin filter, discard flow through, and replace spin filter.

19. Centrifuge again for 30 seconds at 13,000 x g.

20. Carefully remove spin filter and transfer to a new tube without it coming in contact with the wash.

21. Add 200µl of Solution B5. To increase yields, incubate for 5 min. at 65°C.

What’s happening: Solution B5 is 10mM Tris. As it passes through the spin filter, the DNA is released (eluted) off the filter and it passes into the collection tube. The DNA is released because it will not stay bound to the spin filter when there is no salt present.

22. Centrifuge 1 min. at 13,000xg.

23. Remove filter unit and close tube lid. Genomic DNA in the tube is now ready to use for any application.

Thank you for choosing the UltraClean™ BloodSpin Kit.

To concentrate DNA, if desired, see Hints on next page.



Hints and Troubleshooting Guide

Concentrating the DNA

Your final volume will be 200 μ l. If this is too dilute for your purposes, add 8 μ l of 5M NaCl and mix. Then add 400 μ l of 100% cold ethanol. Mix. Centrifuge at 13,000 x g for 5 minutes. Decant all liquid. Dry residual ethanol in a speed vac or desiccator or ambient air. Resuspend precipitated DNA in desired volume.

Amount to process

200 μ l of Whole blood (or Bone Marrow).

Low recovery

If you did not mix sample well after adding Solution B1, you will get incomplete lysis and yields will be lower. Be sure to vortex well at step 4.

DNA floats out of well when loaded on a gel

Residual Solution B4 in the final sample. Prevent this by being careful in step 19 not to transfer liquid onto the bottom of the spin filter basket. Ethanol precipitate to remove residues of Solution B4.



Other quality products 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
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
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: Toll free 1-800-606-6246, or 1-760-929-9911 email: technical@mobio.com



Contact Information

Phone MO BIO Laboratories, Inc. Toll Free 800-606-6246, or 760-929-9911

Email: technical@mobio.com

Fax: 760-929-0109

Mail: MO BIO Laboratories, Inc, 2746 Loker Ave West, Carlsbad, CA 92010

Ordering Information

Direct: Phone MO BIO Laboratories, Inc. Toll Free 800-606-6246, or 760-929-9911

Email: orders@mobio.com

Fax: 760-929-0109

Mail: MO BIO Laboratories, Inc, 2746 Loker Ave West, Carlsbad, CA 92010

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