



UltraClean™ Mega BloodSpin™ Kit

Catalog # 12210-10
10 preps
Instruction Manual

Introduction

Use this kit for isolating Genomic DNA from 10ml 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 sparks.

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

Not supplied: 100% ethanol (333.2 ml), RNase A (20 mg/ml),
Centrifuge (2500 x g) capable of spinning 50ml tubes. 65°C water bath, Pipets (1ml-20 ml), vortex

Kit Contents: Sufficient reagents for 10 preps of 10 ml.

<u>Component</u>	<u>Amount</u>	<u>Description</u>
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Proteinase K*	62.5mg	Proteinase K*
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***Before starting protocol, add 11.0 ml of distilled water to lyophilized Proteinase K**

Solution B1	110 ml	Guanidine HCl/Tween solution
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Solution B2	0 ml	Add 100 ml 100% Ethanol to bottle provided.
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Solution B3	96.8 ml	Wash concentrate/Guanidine HCl solution (before use, add 123.2ml of 100% ethanol)
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Solution B4	110 ml	Wash concentrate/Tris, NaCl. (before use, add 110ml of 100% ethanol)
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Solution B5	110 ml	10 mM Tris-HCl.
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Spin Filters	10	Spin filter basket in a 50 ml tube.
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50 ml tubes	30	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.

Version 01092007

Technical information: Toll free 1-800-606-6246, or 1-760-929-9911 email: technical@mobio.com



Experienced User Protocol

Please wear gloves at all times

1. **Before first use see “Kit Contents” to prepare Proteinase K, Solution B2, B3, and B4.**
2. **Add 10ml of whole blood to 1ml of Proteinase K in a 50 ml tube (provided).**
3. (optional: RNase A can be added at this step. Use 100µl of 20mg/ml.)
4. **Add 10ml of Solution B1.**
5. Bump vortex for 15 seconds.
6. Incubate at 65°C for 10 minutes.
7. Centrifuge briefly to remove any drops from the top of the lid.
8. **Add 10ml of Solution B2 (100% ethanol)** and vortex for 15 seconds.
9. Centrifuge briefly to remove any drops from the top of the lid.
10. **Transfer 20ml** of mixture to the spin filter.
11. Centrifuge 3 minutes at 2500 x g.
12. **Add remaining mixture** to spin filter and centrifuge 3 minutes at 2500 x g.
13. Transfer spin filter to new 50ml tube (provided).
14. Make sure ethanol was added to Solution B3 before first use.
15. **Add 20ml of Solution B3** to spin filter.
16. Centrifuge 3 minutes at 2500 x g.
17. Remove spin filter, dump flow through, and replace spin filter.
18. Make sure ethanol was added to solution B4 before first use.
19. **Add 20ml of Solution B4** to spin filter.
20. Centrifuge 3 minutes at 2500 x g.
21. Remove spin filter, dump flow through, and replace spin filter.
22. Centrifuge again for 3 minutes at 2500 x g.
23. **Carefully remove spin filter** and transfer to a new tube without it coming in contact with the wash solution.
24. **Add 10ml of Solution B5.** To increase yields, incubate for 5 minutes at 65°C.
25. Centrifuge 3 minutes at 2500 x g.
26. Remove filter unit and close tube lid. Genomic DNA in tube is now ready to use for any application.

To concentrate the DNA see the Additional Information Section.

Thank you for choosing the UltraClean™ Mega BloodSpin™ Kit.



Detailed Protocol (Describes each step)

Please wear gloves at all times

1. **See “Kit Contents” before starting to prepare Proteinase K, Solution B2, B3, and B4.**

What’s happening: The Proteinase K solution requires reconstituting the powdered Proteinase K with distilled water before using it the first time. Solutions B2, B3, and B4 require the addition of 100% ethanol before use; the ethanol was excluded from these solutions for shipping reasons.

2. **Add 10ml of whole blood to 1ml of Proteinase K in a 50 ml tube (provided).**

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 100µl 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 10ml 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 10ml of Solution B2 (100% ethanol) 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 20ml of mixture to the spin filter.**

11. **Centrifuge 3 minutes at 2500 x g.**

12. **Add remaining mixture to spin filter and centrifuge 3 minutes at 2500 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.

13. **Transfer spin filter to new 50ml tube (provided).**

14. **Make sure ethanol was added to Solution B3 before first use.**

15. **Add 20ml 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.

16. **Centrifuge 3 minutes at 2500 x g.**

17. **Remove spin filter, dump flow through, and replace spin filter.**

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

18. **Make sure ethanol was added to solution B4 before first use.**

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19. **Add 20ml 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.

20. Centrifuge 3 minutes at 2500 x g.

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

22. Centrifuge again for 3 minutes at 2500 x g.

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

24. **Add 10ml of Solution B5.** To increase yields, incubate for 5 minutes 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.

25. Centrifuge 3 minutes at 2500 x g.

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

To concentrate the DNA see the Additional Information Section.

Thank you for choosing the Ultra Clean™ Mega BloodSpin™ Kit.



Additional Information

Concentrating the DNA

The final volume will be 10ml. If this is too dilute for your purposes, add 1 ml of 5M NaCl and mix. Then add 20ml of 100% cold ethanol. Mix. Ice for an hour. Centrifuge at 2500 x g for 30 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

10ml 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 5.

DNA floats out of well when loaded on a gel

Residual Solution B4 in the final sample. Prevent this by being careful in step 23 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
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

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Contact Information

Technical Information

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