



UltraClean™ Water DNA Isolation Kit (0.45 µm)

Catalog # 14800-25
25 preps
Instruction Manual

Introduction

Use this kit for isolating microbial DNA from water samples.

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.

Equipment required:

Centrifuge (15 ml; 50 ml) 2500 x g
Pipettor (volumes required 50 µl – 200 µl, 100µl - 1000µl,
Up to 10 ml), vortexer, Vacuum Filtration System.

Kit Contents

<u>Description</u>	<u>Amt.</u>
Water Bead Tubes (15 ml tubes contain 5 gm of Beads)	25
Bead solution	110 ml
Solution WD1	15 ml
Solution WD2	20 ml
Solution WD3	250 ml
Solution WD4	3x32 ml
Solution WD5	83 ml
Water filters (0.45µm pore size)	25
Water Filter Adapter	1
Spin filter units in 50 ml tubes	25
Centrifuge tubes (15 ml)	50
Collection Tubes (50ml)	25

Kit Storage

Room temperature

Make sure the 15 ml Bead Solution screw cap tubes rotate freely in your centrifuge without rubbing. Do not spin the bead tubes in excess of 6000 rpm.

WARNING: Solution WD4 contains ethanol, it is flammable.

Version: 06262006

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



Protocol

Please wear gloves at all times

1. Filter the water samples using Water filters provided. Use the filter adapter provided in your filtration assembly. The volume of water you filter depends on the microbial load and turbidity of the water sample. (Please see Types of Water Samples in the Hints section of Instruction Manual)
2. Aseptically remove the filter membrane. The 100 ml upper portion of the filter cup is easily removed from the catch reservoir by snapping it off.
3. Use sterile forceps to pick up the white filter membrane.
4. Insert the filter into a Water Bead Tube.
5. **Add 4 ml of Bead Solution** to the Water Bead Tube.
6. Vortex for 30 seconds.
7. **Add 500 µl of Solution WD1** to each Water Bead tube and vortex it for 30 seconds to mix.
8. Secure Water Bead tubes horizontally to a Mo Bio Vortex adapter Catalog number 13000-V1-15 for a Genie 2 Vortex, or catalog number, 13000-LV2-15, for a Labnet vortex. Alternately, secure on a flatbed vortex pad with tape.
9. Vortex at maximum speed for 10 minutes. Warning: Tape can become loose and samples may not be efficiently vortexed. We strongly recommend using the Mo Bio Vortex adapter.
10. Centrifuge the tubes at 2500 x g for 1 minute.
11. Transfer the supernatant to a clean 15 ml centrifuge tube (provided). **Note:** Expect about 3.4 ml of supernatant.
12. **Add 600 µl of Solution WD2** to the supernatant.
13. Vortex 5 seconds.
14. Incubate at 4°C for 5 minutes.
15. Centrifuge the tubes for 4 minute at 2500 x g.
16. Avoiding the pellet, transfer the entire volume of supernatant to another clean 15 ml tube (provided). You can expect approximately 4 ml in volume.
17. **Add 8 ml of Solution WD3** to the supernatant and vortex 5 seconds.
18. Load supernatant onto 50 ml spin filter tubes and centrifuge at 2500 x g for 2 minutes.
19. Discard the flow through.
20. **Add 3 ml of Solution WD4** and centrifuge for 3 minutes at 2500 x g.
21. Discard the flow through.
22. Centrifuge again for 5 minutes at 2500 x g.
23. Being careful not to splash liquid on the filter basket, place spin filter in a new 50 ml tube (provided).
24. **Add 3 ml of Solution WD5** to the center of the white filter membrane.
25. Centrifuge the tubes for 2 minutes at 2500 x g.
26. Discard spin filter. DNA in the tube is now ready for any application. No further steps are required.

We recommend storing DNA frozen (-20°C). Solution WD5 contains no EDTA.

Thank you for choosing the UltraClean™ Water DNA Isolation Kit.



Detailed Protocol (describes “What’s happening” in each step)

Please wear gloves at all times

1. Filter the water samples using Water filters provided. Use the filter adapter provided in your filtration assembly. The volume of water you filter depends on the microbial load and turbidity of the water sample. (Please see Types of Water Samples in the Hints section of Instruction Manual).

What’s happening: Microorganisms are trapped on the 0.45 micron filter.

2. Aseptically remove the filter membrane. The 100 ml upper portion of the filter cup is easily removed from the catch reservoir by snapping it off.
3. Use sterile forceps to pick up the white filter membrane.
4. Insert the filter into a Water Bead Tube.
5. **Add 4 ml of Bead Solution** to the Water Bead Tube.

What’s happening: Bead Solution begins the cell lysis.

6. Vortex for 30 seconds.
7. **Add 500 µl of Solution WD1** to each Water Bead tube and vortex it for 30 seconds to mix.

What’s happening: Solution WD1 is a strong lysing reagent that includes a detergent to help break cell walls.

8. Secure Water Bead tubes horizontally to a Mo Bio Vortex adapter Catalog number 13000-V1-15 for a Genie 2 Vortex, or catalog number, 13000-LV2-15, for a Labnet vortex. Alternately, secure on a flatbed vortex pad with tape.

9. Vortex at maximum speed for 10 minutes. Warning: Tape can become loose and samples may not be efficiently vortexed. We strongly recommend using the Mo Bio Vortex adapter.

What’s happening: Vortexing will break apart the filter membrane that contains trapped cells. The mechanical action of bead beating aids in cell lysis.

10. Centrifuge the tubes at 2500 x g for 1 minute.

What’s happening: Broken pieces of filter membrane are removed at this step.

11. Transfer the supernatant to a clean 15 ml centrifuge tube (provided). **Note:** Expect about 3.4 ml of supernatant.

12. **Add 600 µl of Solution WD2** to the supernatant.

13. Vortex 5 seconds.

14. Incubate at 4°C for 5 minutes.

What’s happening: The combination of Solution WD2 and cooling precipitates proteins and unwanted cell debris.

15. Centrifuge the tubes for 4 minute at 2500 x g.

16. Avoiding the pellet, transfer the entire volume of supernatant to another clean 15 ml tube (provided). You can expect approximately 4 ml in volume.

What’s happening: The supernatant contains unpurified microbial DNA.

17. **Add 8 ml of Solution WD3** to the supernatant and vortex 5 seconds.

What’s happening: Solution WD3 is a binding salt solution.

18. Load supernatant onto 50 ml spin filter tubes and centrifuge at 2500 x g for 2 minutes.

What’s happening: Microbial DNA is bound to the spin filter under high salt conditions.

19. Discard the flow through.

20. **Add 3 ml of Solution WD4** and centrifuge for 3 minutes at 2500 x g.

What’s happening: Solution WD4 washes the DNA that is bound to the spin filter. Solution WD4 is about 50% ethanol. The ethanol keeps the microbial DNA bound to the filter as impurities are washed away.



21. Discard the flow through.

22. Centrifuge again for 5 minutes at 2500 x g.

What's happening: This second centrifuge step removes any traces of wash Solution WD4.

23. Being careful not to splash liquid on the filter basket, place spin filter in a new 50 ml tube (provided).

24. **Add 3 ml of Solution WD5** to the center of the white filter membrane.

25. Centrifuge the tubes for 2 minutes at 2500 x g.

What's happening: Solution WD5 is 10mM Tris. As it passes through the spin filter, the microbial 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.

26. Discard spin filter. DNA in the tube is now ready for any application. No further steps are required.

We recommend storing DNA frozen (-20°C). Solution WD5 contains no EDTA.

Thank you for choosing the UltraClean™ Water DNA Isolation Kit.



Hints and Troubleshooting Guide

Types of Water Samples

A. Clear Water Samples

Water samples may vary from clear to highly turbid. Larger volumes of clear water can be processed because there is less chance of filter clogging. Potable drinking water will generally allow for very high volumes depending on quality and particulate count. In most cases, 100 ml to 10 liters can be processed. Some users report processing even higher volumes.

B. Turbid Samples

Turbid samples or samples with high levels of suspended solids or sediments will tend to clog the water filter provided. This is because the pore size of the filter supplied is only 0.45 microns. A method of filtering turbid samples generally involves stacking filters with larger pore sizes on top of the 0.45 micron filter provided. A common set up is to stack a sterile 1 micron paper filter just above the 0.45 micron filter. Then layer a sterile 3-8 micron paper filter on top of the 1 micron filter. This layering will filter out large debris and allow the 0.45 micron filter to trap microorganisms. The layered filter system can be washed with sterile water or sterile phosphate buffer to knock down some of the trapped microorganisms on the larger pore size filters. Although this is not 100% efficient, it will increase the overall yield of microbial DNA.

Concentrating the DNA

Your final volume will be 3 ml. If this is too dilute for your purposes, add 300 μ l of 5M NaCl and mix. Then add 6 ml of 100% ice-cold ethanol. Mix. Centrifuge at 2500 x g for 20 minutes. Decant all liquid. Dry residual ethanol in a speed vac or dessicator or ambient air. Resuspend precipitated DNA in desired volume.

Trouble amplifying DNA by the PCR reaction

Some water samples are high in humic acid. Humics are a class of chemical compounds known to inhibit PCR by reducing DNA polymerase activity. The removal of humics during the DNA isolation procedure, depends on the efficiency of washing after binding DNA to the spin filter. It may be necessary to wash more than once to reduce humic acid contamination in the final sample. If the eluted DNA has a brown coloration, it may contain humic acid.

Removing humic acid contamination from final sample

If the DNA recovered at the end of the isolation procedure has a brown color, it may contain humic acid. In general, PCR may be difficult with this DNA. Dilute DNA 10 fold prior to attempting PCR.

DNA floats out of well when loaded on a gel

You may have inadvertently transferred some residual Solution WD4 into the final sample. Prevent this by being careful in step 13 not to transfer liquid onto the bottom of the spin filter basket. Ethanol precipitation is the best way to remove residues of Solution WD4. (See concentrating DNA above)

Storing DNA

DNA is eluted in Solution WD5 (10mM Tris) therefore it must be stored at -20°C or it may degrade. DNA can be eluted in TE but the EDTA may inhibit reactions such as PCR and automated sequencing.



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

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