UltraClean®-htp
96 Well Microbial DNA Isolation Kit

Catalog No.	Quantity	Total Purifications
10196-4	4 Preps	384

Instruction Manual

Please recycle

Version: 08112016

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Introduction

The UltraClean®-htp 96 Well Microbial DNA Isolation Kit is designed to isolate high-quality genomic DNA from microorganisms. A variety of microorganisms including bacterial spores and fungal types have been tested successfully with this kit.

Protocol Overview

Microbial cells are added to a bead beating plate containing beads, bead solution and lysis solution. The principal is to lyse the microorganisms by a combination of heat, detergent, and mechanical force against specialized beads. The cellular components are lysed by mechanical action using a 96 Well Plate Shaker. From the lysed cells, the released DNA is bound to a silica spin plate. The plate is washed, and the DNA is recovered in certified DNA-free Tris buffer.

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

Other Related Products	Catalog No.	Quantity
96 Well Plate Shaker	11996	1 unit
Plate Adapter Set	11990	1 set

Equipment Required

Centrifuge capable of spinning two 96 Well blocks stacked (13 cm x 8 cm x 5.5 cm) at 4500 x *g*

Note: If you have a centrifuge with a maximum speed less than 4500 x *g* see the Hints and Troubleshooting Guide.

Multi-channel Pipettor (volumes required 50 µl - 650 µl)
Mechanical Shaker that shakes 96 Well Blocks and Adapters (MO BIO Catalog# 11996 & 11990)
Vortex with 3 inch platform

Optional Equipment

Reagent reservoirs
Vacuum pump (MO BIO Catalog# 11998)
Vacuum manifold (MO BIO recommends Axygens' AxyVac Vacuum Manifold Catalog# AP-VM)

Kit Contents

	Kit Catalog # 10196-4	
Component	**Catalog #**	**Amount**
Bead Plates	10196-4-BP	4
Bead Plate Sealing Mats	10196-4-SM	4
MicroBead Solution	10196-4-BS	127 ml
Solution MD1	10196-4-1	26 ml
Solution MD2	10196-4-2	43 ml
Solution MD3	10196-4-3	340 ml
Solution MD4	10196-4-4	330 ml
Solution MD5	10196-4-5	43 ml
Spin Plates	10196-4-SP	4
2 ml Collection Plates	10196-4-2CP	12
1 ml Collection Plates	10196-4-1CP	4
0.5 ml Collection Plates	10196-4-0.5CP	4
Microplates	10196-4-MP	4
Centrifuge Tape	10196-4-CT	24
Sealing Tape	10196-4-ST	12
Round Well Mats	10196-4-ESM	4

Kit Storage

Kit reagents and components should be stored at room temperature (15-30°C).

Precautions:

Please wear gloves when using this product. Avoid all skin contact with kit reagents. 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 at www.mobio.com. Reagents labeled flammable should be kept away from open flames and sparks.

WARNING: Solution MD4 contains ethanol, it is flammable.

IMPORTANT NOTE FOR USE: Shake to mix Solution MD3 before use

Combination of Vacuum and Centrifugation Protocol

This protocol assumes you will be processing 192 samples (2 x 96 well preps). If you plan to process less than this number, divide your samples between two plates evenly so that you always have a balance. See Hints and Troubleshooting Guide.

1. Dispense the liquid culture in to the 2 ml Collection Plate, and cover with Sealing Tape. Centrifuge at 4500 x *g* for 12 minutes.
2. Discard tape and remove all supernatant.
3. **Add 300 µl of MicroBead Solution** and apply a new piece of Sealing Tape. Resuspend the cell pellet in the MicroBead Solution by vortexing.
4. Centrifuge the **Bead Plate** for 3 minutes at 4500 x *g* to bring all beads down to the bottom of the wells.
5. Remove and discard the Bead Plate Sealing Mat from the Bead Plate and transfer resuspended cells into the **Bead Plate**.
6. (Check Solution MD1). If precipitated, heat to 60°C until dissolved.
7. **Add 60 µl of Solution MD1.** Seal Bead Plate with a new Bead Plate Sealing Mat (provided).
8. Place Bead Plate with Bead Plate Sealing Mat securely fastened, on the 96 Well Plate Shaker (Catalog# 11996).
   Note: The final order of all components is: Adapter plate, Bead Plate Sealing Mat, Bead Plate and Adapter plate.
9. Shake at speed 20 for 5 minutes, remove plates and re-orient them so that the side closest to the machine body is now furthest from the machine body and shake again at speed 20 for 5 minutes.
10. Centrifuge the Bead Plates for 6 minutes at 4500 x *g.*
11. Remove and discard Bead Plate Sealing Mat. Transfer the supernatant to a clean 1 ml Collection Plate.
    Note: Supernatant may still contain some beads.
12. **Add 100 µl of Solution MD2** and place Sealing Tape onto plate. Vortex for 5 seconds. Incubate 4°C for 10 minutes.
13. Centrifuge the plate for 9 minutes at 4500 x *g*.
14. Avoiding the pellet, transfer supernatant to a 2 ml Collection Plate.
15. Remove the top portion of the vacuum manifold and place a new 2 ml Collection Plate in the bottom of the vacuum manifold. Replace top of manifold. Now place Spin Plate onto top of manifold. Turn the vacuum pump on.
    Note: If you are using the Axygen AP-VM, use the Waste Reservoir provided with the manifold in place of the 2 ml Collection Plate.
16. To maximize pipet tip efficiency, please process 8 samples (1 row) at a time using a multi channel pipettor.
17. Shake to mix Solution MD3 before use. **Add 800 µl of Solution MD3** to the wells of the 2 ml Collection Plate containing the supernatant.
18. Pipet up and down to mix.
19. Load 650 µl from the Collection Plate onto the first row of the Spin Plate and apply vacuum. After supernatant has passed through, load remaining 650 µl onto Spin Plate so that the entire volume from the Collection Plate has been loaded onto the Spin plate. Repeat this step until all samples have been processed. Turn off the vacuum pump.
20. Remove the Spin Plate from the manifold and set aside, discard the flow through from the 2 ml Collection Plate or Waste Reservoir in the bottom of the manifold and then place it back in the manifold. Place the Spin Plate back on the manifold, and turn the vacuum on.
21. **Add 400 µl of Solution MD4** to each well of the Spin Plate.
22. After the first volume of MD4 has passed through the Spin Plate, **Add another 400 µl volume of MD4.**

23. After entire volume of Solution MD4 has passed through the Spin Plate, turn the vacuum off.
24. Apply Centrifuge Tape to the Spin Plate to cover the wells. Place a 0.5 ml Collection Plate under the Spin Plate.
25. Centrifuge for 6 minutes at 4500 x *g*. Carefully place Spin Plate on a MicroPlate being careful not to splash any residual MD4 onto Spin Plate.
26. Discard supernatant and 0.5 ml Collection Plate.
27. Remove Centrifuge Tape and discard.
28. Allow to air dry for 10 minutes at room temperature.
29. **Add 100 µl of Solution MD5** to the center of the white filter membrane of Spin Plate.
30. Apply Centrifuge Tape to the Spin Plate to cover the wells.
31. Centrifuge for 3 minutes at 4500 x *g*.
32. Cover wells of Microplate with Round Well Mat provided.
33. DNA in the Microplate is now application ready. No further steps are required.

We recommend storing DNA frozen (-20°C). Solution MD5 contains no EDTA.

Thank you for choosing the UltraClean®-htp 96 Well Microbial DNA Isolation Kit.

Centrifugation Protocol

This protocol assumes you will be processing 192 samples (2 x 96 well preps). If you plan to process less than this number, divide your samples between two plates evenly so that you always have a balance. See Hints and Troubleshooting Guide.

1. Dispense the liquid culture in to the 2 ml Collection Plate, and cover with Sealing Tape. Centrifuge at 4500 x *g* for 12 minutes.
2. Discard tape and remove all supernatant.
3. **Add 300 µl of MicroBead Solution** and apply a new piece of Sealing Tape. Resuspend the cell pellet in the MicroBead Solution by vortexing.
4. Centrifuge the **Bead Plate** for 3 minutes at 4500 x *g* to bring all beads down to the bottom of the wells.
5. Remove and discard the Bead Plate Sealing Mat from the Bead Plate and transfer resuspended cells into the **Bead Plate**.
6. (Check Solution MD1). If precipitated, heat to 60°C until dissolved.
7. **Add 60 µl of Solution MD1.** Seal Bead Plate with a new Bead Plate Sealing Mat (provided).
8. Place Bead Plate with Bead Plate Sealing Mat securely fastened, on the 96 Well Plate Shaker (MO BIO Catalog# 11996).
   Note: The final order of all components is: Adapter plate, Bead Plate Sealing Mat, Bead Plate and Adapter plate.
9. Shake at speed 20 for 5 minutes, remove plates and re-orient them so that the side closest to the machine body is now furthest from the machine body and shake again at speed 20 for 5 minutes.
10. Centrifuge the Bead Plates for 6 minutes at 4500 x *g*.
11. Remove and discard Bead Plate Sealing Mat. Transfer the supernatant to a clean 1 ml Collection Plate.
    Note: Supernatant may still contain some beads.
12. **Add 100 µl of Solution MD2** and place Sealing Tape onto plate. Vortex for 5 seconds. Incubate 4°C for 10 minutes.
13. Centrifuge the plate for 9 minutes at 4500 x *g*.
14. Avoiding the pellet, transfer supernatant to a 2 ml Collection Plate.
15. Shake to mix Solution MD3 before use. **Add 800 µl of Solution MD3** to the wells of the 2 ml Collection Plate containing the supernatant.
16. Pipet up and down to mix.
17. Place Spin Plate onto a new 0.5 ml Collection Plate.
18. Load approximately 650 µl onto the Spin Plate. Apply Centrifuge Tape.
19. Centrifuge for 3 minutes at 4500 x *g*.
20. Discard flow through from the 0.5 ml Collection Plate and replace same 0.5 ml Collection Plate beneath the Spin Plate. Discard Centrifuge Tape.
21. Repeat steps 18 to 20 until all supernatant has been processed.
22. **Add 400 µl of Solution MD4** to the wells of the Spin Plate, apply Centrifuge Tape and centrifuge at 4500 x *g* for 3 minutes.
23. Discard Centrifuge Tape and flow through. Replace Spin Plate onto same 0.5 ml Collection Plate. **Add another 400 µl volume of MD4.** Apply a new piece of Centrifuge Tape. Centrifuge for 3 minutes at 4500 x *g*.
24. Discard flow through. Replace Spin Plate onto same 0.5 ml Collection Plate.
25. Centrifuge for 6 minutes at 4500 x *g*. Carefully place Spin Plate on a MicroPlate being careful not to splash any MD4 onto Spin Plate.
26. Discard supernatant and 0.5 ml Collection Plate.
27. Remove Centrifuge Tape and discard.
28. Allow to air dry for 10 minutes at room temperature.

29. **Add 100 µl of Solution MD5** to the center of the white filter membrane of Spin Plate.
30. Apply Centrifuge Tape to the Spin Plate to cover the wells.
31. Centrifuge for 3 minutes at 4500 x *g*.
32. Cover wells of the Microplate with Round Well Mat provided.
33. DNA in the Microplate is now application ready. No further steps are required.

We recommend storing DNA frozen (-20°C). Solution MD5 contains no EDTA.

Thank you for choosing the UltraClean®-htp 96 Well Microbial DNA Isolation Kit.

Hints and Troubleshooting Guide

Processing Less Than 192 Samples (less than 2 full plates)

This protocol assumes you will be processing 192 samples (2-96 well preps). If you plan to process less than this number, divide your samples between two plates evenly.

Distributing samples between two plates:

Balance the number of samples so centrifugation steps do not damage your centrifuge. It is best to match the total number of samples per plate as well as the orientation. For example, if you use wells A1-A12 in one plate, use those same wells in the second plate.

Multi-Channel pipettors:

The use of a multi-channel pipettor is advised for maximum efficiency. Most multi-channel pipettors are made to pipet multiples of 8 or 12 samples at a time. Try to purchase one that can pipet a broad range of volumes. (Volumes required 50 µl - 650 µl).

Mark used wells:

Be sure to mark all used wells to prevent reusing wells and cross contamination.

Using the remaining wells of a previously processed plate:

Be sure to tap the plate several times on the lab bench to force any beads to the bottom of the deep well plate before re-using a plate.

Vacuum Hints

If a vacuum step seems to be taking a long time, turn off the vacuum source. Lift the filter plate off the vacuum to release any back pressure. Replace the filter plate and turn the vacuum source back on. Be sure there are no air leaks around the plate. If slow vacuum continues, you can centrifuge the filter plate as an alternative.

Centrifuge with a Maximum Speed Less Than 4500 x g

Multiply the protocol time and speed to determine total x g. Divide the total by the maximum speed of your centrifuge (round up if necessary). This will be the number of minutes your centrifuge will need to run to achieve the appropriate overall force.

Example: 10 minutes at 4500 x *g* = 45000.

If your centrifuge has a maximum speed of 2500 x *g*, divide 45000 ÷ 2500 = 18 minutes of centrifugation.

DNA Floats Out of Well When Loaded on a Gel

You may have inadvertently transferred some residual Solution MD4 into the final sample. Ethanol precipitation is the best way to remove residues of Solution MD4.

Storing DNA

DNA is eluted in Solution MD5 (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.

Cells are Difficult to Lyse

If cells are difficult to lyse, a 10 minute incubation at 70°C, after adding Solution MD1, can be performed. Follow by continuing with protocol step 8.

Contact Information

Technical Support:
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For the distributor nearest you, visit our web site at www.mobio.com/distributors

Products recommended for you

For a complete list of products available from MO BIO Laboratories, Inc., visit www.mobio.com

Description	Catalog No.	Quantity
96 Well Plate Shaker	11996	1 unit (120 V)
Plate Adapter Set	11990	1 set
PowerMag® Microbial DNA Isolation Kit	27200-4	4 x 96 preps
PowerFood® Microbial DNA Isolation Kit	21000-100	100 preps
PowerLyzer® UltraClean® Microbial DNA Isolation Kit	12255-50	50 preps
UltraClean® Microbial DNA Isolation Kit	12224-50 12224-250	50 preps 250 preps
PowerMicrobial® Maxi DNA Isolation Kit	12226-25	25 preps
PowerLyzer® 24 Bench Top Bead-Based Homogenizer	13155	1 unit