



UltraClean-htp™ 96 Well Plasmid Prep Kit

Catalog # 12396-12
(12) 96 Well Preps

Instruction Manual

Introduction

Use this kit for isolating plasmid DNA grown in *E. coli* host strains. There is an increased yield by using high nutrient media such as Terrific Broth, TB DRY™, and 2X YT. TB DRY™ Mo Bio Catalog #12105. TB DRY™ is a single powder formulation of Terrific Broth. Just autoclave, and use. Two protocols are available for this kit. One is an all centrifugation steps protocol (recommended). The other is a vacuum protocol with some centrifugation steps. In both protocols it is important to check your centrifuge and rotor to be sure they can accommodate all the plates used in this kit. Each protocol has a "Before you start" section to help you with this.

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:

Centrifuge capable of spinning two 96 Well blocks stacked (13 cm x 8 cm x 5.5 cm) at 2500 x g,
Multi-channel Pipettor (volumes required 50 µl - 700 µl)
Vortex with 3 inch platform

Optional equipment required:

Vacuum pump, Vacuum manifold Mo Bio Catalog number: 11997

Kit Contents

<u>Description</u>	<u>Amt.</u>
Solution 1	129 ml
Solution 2	255 ml
Solution 3A	381 ml
Solution 3B	762 ml
Solution 4	30 x 30 ml
Solution 5	129 ml
Spin Plates	12
Filter Plates	12
2 ml Collection Plates	24
1 ml Collection Plates	12
0.5 ml Collection Plates	12
Microplates	12
Sealing Tape	72
Centrifuge Tape	72
Elution Sealing Mat	12

Kit Storage: Room temperature.

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



Protocol 1 Centrifuge Protocol

Before you start: Please see precautions on previous page.

There are several things which will make this protocol more efficient to use.

- ◆ First, be sure to measure the centrifuge and rotor you plan to use and be sure they will accommodate the plates used in this kit. For this Centrifuge Protocol it is best to stack a Spin Plate on top of a 0.5 ml Collection Plate. Place this in the plate holder rotor. DO NOT start the centrifuge or possible injury or centrifuge damage may occur. Turn the centrifuge by hand slowly and be sure the stacked plates will clear the rotor and centrifuge.
- ◆ Make sure you have a multi-channel pipettor that can accommodate all the required volumes (50µl-700µl).
- ◆ 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 so that you always have a balance. See Hints section.

Protocol

1. Grow cells (plasmid culture) to a typical density of $A_{600} = 2.0$ or higher.
2. Dispense 2 ml of an overnight culture into the 2 ml Collection Plate and seal the blocks with Sealing Tape. Centrifuge the blocks at 2500 x g for 15 minutes, remove the Sealing Tape and discard.
3. Discard supernatant by decanting and retaining the cell pellet.
4. Leave the 2 ml Collection Plates inverted on a paper towel for 5 minutes to remove the remaining liquid media. Alternatively, you can tap them gently on the paper towel.
5. **Add 100µl of Solution 1** to the 2 ml Collection Plate.
6. Seal the wells with Sealing Tape (Do not use Centrifuge tape for this step).
7. Vortex vigorously on a vortex with a 3 inch rubber platform until the cell pellet is completely resuspended. (Hint: Move the Plate back and forth (side ways) on the vortexer, for efficient and quick resuspension.)
8. **(Check Solution S2)**. If precipitated, heat to 60°C until dissolved. Use this reagent between 20-35°C.
9. Remove the Sealing Tape and discard.
10. **Add 200µl of Solution 2** and seal with new Sealing Tape. (IMPORTANT: Be sure to form a good seal on all wells or they will leak).
11. Invert the 2 ml Collection Plate upside down once and incubate at room temperature for 5 minutes. (Note: Do not incubate more than 5 minutes at room temperature.)
12. Remove and discard Sealing Tape.
13. **Add 300 µl of Solution 3A** and seal with new Sealing Tape.
14. Invert upside down once.
15. Place a Filter Plate on top of a new 0.5 ml Collection Plate and transfer all of the lysate in the 2 ml Collection Plate to the Filter Plate. Apply a new piece of Centrifuge Tape to the top of the Filter Plate.
16. Centrifuge the stacked plates (Filter Plate on top of 0.5 ml Collection Plate) for 5 minutes at 2500 x g.
17. Discard Filter Plate.
18. Transfer the filtered lysate to a new 1 ml Collection Plate.
19. **Add 600 µl of Solution 3B** to the 1 ml Collection Plate containing the lysate.
20. Mix by pipetting up and down 5 times.
21. Place the same 0.5 ml Collection Plate beneath a Spin Plate.
22. Load 600 µl from the 1 ml Collection Plate into the Spin Plate. Apply Centrifuge Tape to the top of the Spin Plate. Centrifuge the stacked Spin Plate/ 0.5 ml Collection Plate for 3 minutes at 2500 x g.
23. Discard the flow through from the 0.5ml Collection Plate.
24. Replace the Spin Plate onto the same 0.5 ml Collection Plate.
25. Remove and discard the Centrifuge Tape from the Spin Plate.
26. Transfer the remaining volume of lysate to the Spin Plate and apply a new piece of Centrifuge Tape.

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27. Centrifuge the stacked Spin Plate/ 0.5 ml Collection Plate for 3 minutes at 2500 x g.
28. Discard the flow through and reuse the 0.5 ml Collection Plate for the following wash steps.
29. Place Spin Plate on the same 0.5 ml Collection Plate.
30. Remove and discard Centrifuge Tape.
31. **Add 350 µl of Solution 4** to the Spin Plate.
32. Apply a new piece of Centrifuge Tape.
33. Centrifuge for 5 minutes at 2500 x g.
34. Discard flow through in the 0.5 ml Collection Plate.
35. **Add another 350 µl of Solution 4** to the Spin Plate.
36. Apply a new piece of Centrifuge Tape.
37. Centrifuge for 5 minutes at 2500 x g to remove any residual Solution 4.
38. Discard flow through.
39. Re-place 0.5 ml Collection Plate beneath Spin Plate.
40. Centrifuge again for 5 minutes at 2500 x g.
41. Carefully transfer Spin Plate to a Microplate.
42. Discard Centrifuge Tape.
43. **Add 50µl of Solution 5** to the Spin Plate.
44. Apply a new piece of Centrifuge Tape to the Spin Plate.
45. Centrifuge for 5 minutes at 2500 x g.
46. Remove and discard Centrifuge Tape.
47. **Add another volume of 50µl of Solution 5** to the Spin Plate. (This will result in two 50µl volume elutions combined into the same Microplate.)
48. Apply Centrifuge Tape.
49. Centrifuge for 5 minutes at 2500 x g.
50. DNA is now collected in Microplate. Remove Spin Plate and discard.
51. Seal Microplate with Elution Sealing Mat.

DNA in the Microplate is now application ready. No further steps are required.
We recommend storing DNA frozen (-20°C). Solution 5 contains no EDTA.

Thank you for choosing the UltraClean 96 Well Plasmid Prep Kit.

Version 03222005



Protocol 2

Combined Vacuum and Centrifuge Protocol

Before you start: Please see precautions on page 1.

There are several things which will make this protocol more efficient to use.

- ◆ First, be sure to measure the centrifuge and rotor you plan to use and be sure they will accommodate the plates used in this kit. For this **Centrifuge and Vacuum** Protocol all that is required is that the 2 ml Collection plate fits your centrifuge and rotor. Place it in the plate holder rotor. **DO NOT** start the centrifuge or possible injury or centrifuge damage may occur. Turn the centrifuge by hand slowly and be sure the plate will clear the rotor and centrifuge.
- ◆ Make sure you have a multi-channel pipettor that can accommodate all the required volumes (50µl-700µl).
- ◆ A vacuum manifold is required for this protocol. We recommend the use of Mo Bio manifold catalog number 11997. If you plan to use another manifold, be sure it is compatible with the plates in this kit.
- ◆ 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 so that you always have a balance. See Hints section.

Protocol

1. Grow cells (plasmid culture) to a typical density of $A_{600} = 2.0$ or higher.
2. Dispense 2 ml of an overnight culture into the 2 ml Collection Plate and seal the blocks with Sealing Tape. Centrifuge the blocks at 2500 x g for 15 minutes, remove the Sealing Tape and discard.
3. Discard supernatant by decanting and retaining the cell pellet.
4. Leave the 2 ml Collection Plates inverted on a paper towel for 5 minutes to remove the remaining liquid media. Alternatively, you can tap them gently on the paper towel.
5. **Add 100µl of Solution 1** to the 2 ml Collection Plate.
6. Seal the wells with Sealing Tape (Do not use Centrifuge tape for this step).
7. Vortex vigorously on a vortex with a 3 inch rubber platform until the cell pellet is completely resuspended. (Hint: Move the Plate back and forth (side ways) on the vortex, for efficient and quick resuspension.)
8. **(Check Solution S2)**. If precipitated, heat to 60°C until dissolved. Use this reagent between 20-35°C.
9. Remove the Sealing Tape and discard.
10. **Add 200µl of Solution 2** and seal with new Sealing Tape. (IMPORTANT: Be sure to form a good seal on all wells or they will leak).
11. Invert the 2 ml Collection Plate upside down once and incubate at room temperature for 5 minutes. (Note: Do not incubate more than 5 minutes at room temperature.)
12. Remove and discard Sealing Tape.
13. **Add 300 µl of Solution 3A** and seal with new Sealing Tape.
14. Invert upside down once.
15. Leave the 2 ml Collection Plate at room temperature while you assemble your vacuum manifold. (IMPORTANT: Mo Bio highly recommends the use of our manifold catalog number 11997. For directions on other manifolds, contact the manufacturer.)
16. Remove the top of the manifold. Place a new 1ml Collection Plate in the bottom region of the vacuum manifold and re-place the top of the manifold.
17. Place a new Filter Plate on the top of the manifold.
18. Go deep into the wells of the 2 ml Collection Plate and remove 300 µl of the neutralized lysate. Load on to the Filter Plate.
19. Seal any **unused** wells with Sealing Tape.
20. Turn on vacuum source.
21. Wait until all 300 µl has passed through the Filter Plate.

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22. Add the remaining volume of lysate from the 2 ml Collection Plate trying to avoid white precipitate. *(Note: Some precipitate may be inadvertently transferred. This will not interfere with final yields or quality of DNA.)*
23. Wait until all remaining liquid has passed through the Filter Plate.
24. Turn off the vacuum source.
25. Discard Filter Plate.
26. Remove the top of the manifold.
27. Remove the 1 ml Collection Plate from the bottom of the manifold and set aside.
28. **Add 600µl of Solution 3B** to the supernatant in the 1 ml Collection Plate and pipet up and down 3-4 times to mix.
29. Remove the top of the manifold.
30. Place a new 2ml Collection Plate in the bottom region of the vacuum manifold.
31. Re-place the top of the manifold.
32. Place the Spin Plate on the top on the manifold.
33. Seal any unused wells with Sealing Tape.
34. Load 700 µl of the filtered lysate from the 1 ml Collection Plate into the Spin Plate.
35. Turn on vacuum source.
36. Wait until all liquid has passed through the Spin Plate.
37. Load all remaining filtered lysate from the 1 ml Collection Plate onto the Spin Plate.
38. Turn off vacuum source.
39. Discard the flow through from the 2 ml Collection Plate and replace same plate in the bottom of the manifold.
40. Re-place the Spin Plate on top of the manifold..
41. **Load 700 µl of Solution 4** into the Spin Plate.
42. Turn on the vacuum.
43. After the entire volume flows through, switch off the vacuum, remove the Spin Plate and GENTLY tap the bottom of the plate (the drip region) on a paper towel to remove the residual Solution 4.
44. Discard liquid from the 2 ml Collection Plate and replace it in the bottom of the manifold.
45. Replace the top of the manifold.
46. Place the spin Plate it back on the vacuum manifold and switch on the vacuum source.
47. Let Spin Plate sit under vacuum source for 10 minutes allowing plate to dry.
48. Turn off vacuum source.
49. Remove the 2 ml Collection Plate from the manifold and discard.
50. Place a Spacer Block in the bottom of manifold. Next, place the Microplate on top of the Spacer in the bottom of manifold. The Microplate should be very close to the drip area of the Spin Plate. Mo Bio manifolds are equipped with this Spacer Block. If you do not have one, a pipet tip rack top might be a good substitute.
51. Replace top of manifold.
52. Replace Spin Plate on top of manifold.
53. **Add 50 µl of Solution 5** to the Spin Plate and turn on the vacuum source for 5 minutes.
54. **Add another 50 µl of Solution 5.** Turn vacuum source on for 5 minutes.
55. Turn off vacuum.
56. Collect Microplate from bottom of vacuum manifold.
57. Seal Microplate with Elution Sealing Mat.

DNA in the Microplate is now application ready. No further steps are required.
We recommend storing DNA frozen (-20°C). Solution 5 contains no EDTA.

Thank you for choosing the UltraClean 96 Well Plasmid Prep Kit.

Version 03222005



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 μ l - 650 μ l).

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

- ◆ Always seal any unused wells when vacuuming. Failure to do so will increase the time it takes for liquids to be pulled through Filter Plates and Spin Plates.
- ◆ 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.

DNA floats out of well when loaded on a gel

You may have inadvertently transferred some residual Solution 4 into the final sample. Ethanol precipitation is the best way to remove residues of Solution 4.

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.

Concentrating the DNA

Your final volume will be 100 μ l. If this is too dilute for your purposes, add 4 μ l of 5M NaCl and mix. Then add 200 μ l of 100% cold ethanol. Mix. Centrifuge at 2500 x g for 25 minutes. Decant all liquid. Dry residual ethanol in a speed vac or desiccator or ambient air. Resuspend precipitated DNA in desired volume.



Amount of culture to process

2 mls of high nutrient media or 5 mls of LB culture. (To get higher yields use TB DRY High nutrient media. Cat #12105)

To combine cells from up to 5 mls of LB culture:

Centrifuge 2 ml of culture for 15 minutes in a 2 ml Collection Plate (provided). Discard supernatant. Repeat until 5 ml of culture has been processed into one Plate.

The cell pellet was not completely resuspended. In this case, a low yield results because clumps of cells do not lyse efficiently. Make sure no clumps exist in the plate before adding Solution 2.



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
Fax: 760-929-0109 Mail: Mo Bio Laboratories, Inc., 2746 Loker Avenue West, Carlsbad, CA 92008

Ordering Information

Direct: Call Mo Bio Laboratories, Inc. Toll free 1-800-606-6246, or 1-760-929-9911

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Fax: 760-929-0109 Mail: Mo Bio Laboratories, Inc. 2746 Loker Avenue West, Carlsbad CA 92008

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