UltraClean™
Standard Mini Plasmid Prep Kit™

Catalog # 12301-50
50 preps

Instruction Manual

Introduction
Use this kit for isolating plasmids 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 is a single powder formulation of Terrific Broth. Just autoclave and use.

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:
Microcentrifuge (10,000 x g), vortex, 80 well microcentrifuge tube rack (optional)
2 Pipettors: It makes things easier to have two pipettors: a P200 set to 50 µl and a P1000 set to 250 µl.

Kit Contents: Sufficient reagents for 50 mini plasmid preps.
Note: Economical 250 prep kit size available. Cat# 12301-250.

Component	Amount	Description
Solution 1:	14 ml	Cell suspension buffer:Tris, EDTA, RNase A
Solution 2:	14 ml	Cell lysis solution: SDS/NaOH.
Solution 3:	20 ml	Binding buffer: KoAcetate/ binding salt
Solution 4:	2 x 30 ml	Wash solution: EtOH, Tris, NaCl.
Solution 5:	3 ml	Elution buffer containing 10 mM Tris-HCl.
Spin Filters	50	Spin filter basket in a 2.0 ml tube.
2.0 ml tubes	50	Microcentrifuge tubes.

Kit Storage
Room temperature for 1 year.

Avoid contact of Solution #3 with bleach or other oxidizers.
WARNING: Solution #4 is flammable.

Detailed Protocol

We highly recommend you read this information if this is your first time using the UltraClean Standard Mini Plasmid Prep Kit.

Please wear gloves at all times

Equipment required:

Microcentrifuge (10,000 x g), vortex, 80 well microcentrifuge tube rack (optional)
2 Pipettors: It makes things easier to have two pipettors: a P200 set to 50 µl and a P1000 set to 250 µl.

1. Grow cells (plasmid culture) to a typical density of A_{600} = 2.0 or higher.
2. Each plasmid prep will require a set of 2 tubes and 1spin filter unit (A plastic spin basket with a white silica membrane sitting inside a 2 ml microcentrifuge tube).
3. Label the caps of each set with an ethanol resistant lab marker. It is convenient to place these sets in a microcentrifuge tube rack in this order: tube, spin filter, tube.
4. Open the first tube in each set, and add up to 2 ml of culture. If you are using high nutrient broth (terrific broth, TB DRY™, 2 x YT, super broth, etc.), use no more than 2 ml of culture per prep. For LB or low nutrient cultures, you can use 2 ml or you can combine cells from up to 5ml of culture. Do this by spinning 2 ml of cells, discarding the supernatant, adding more culture to the same tube and spinning again. Repeat until 5 ml worth of cells have been processed. Note: the yields of many low copy plasmids (containing large inserts), can be drastically increased by growing in high nutrient broth such as TB DRY. (Mo Bio catalog number 12105-1)
5. Orient the microcentrifuge tubes the same way each time you spin them in all the following procedures so that the tube hinge is facing straight out away from the center of the rotor.
6. Centrifuge for 1 minute at 10,000 x g. (usually 15,000 rpm).
   What's happening: The bacterial cells are being forced to the bottom of the tube.
7. Decant the supernatant by inverting the tube and pouring into a waste container. You will need to do another spin to be sure to remove all traces of liquid from the sides of the tube as described in the next 3 steps.
8. Orient tubes with the hinges in same position (hinges pointing straight out from center of rotor).
9. Centrifuge 5 seconds at 10,000 x g.
10. Remove **<u>all</u>** visible liquid with a narrow pipet tip. **Removing all liquid at this step is critical. Using a small bore pipet tip helps remove all traces of liquid media.**
    What's happening: The cells have been pelleted and are now separated from the culture growth medium.
11. **Add 250 µl Solution 1** to each cell pellet tube and close the tubes.
12. Resuspend the bacterial pellet by bump vortexing with the vortex set at the highest speed. Bump vortexing means: hold the tube tip on the vortex head for 10 seconds, take it off for 1 second then hold it on the vortex again. Repeat this process for 1 minute. After 1 minute, hold the tube in a horizontal position up to a light and look at it. The liquid will spread from one end of the tube to the other. If you see any clumps of cells, keep bump vortexing until they are gone. It takes a minimum of 1 minute with the vortex at its highest speed to resuspend cells in the 250 µl volume. Do two tubes at a time when processing multiple preps for best efficiency. An alternative procedure is to scrape the tube tips back and forth over the holes of an 80 well microcentrifuge tube rack. This procedure is actually the subject of a paper in *Biotechniques*. Voo, K. S.; Jacobsen, B. M. *BioTechniques 24:240-243, February 1998.*
    What's happening: The bacterial cells are re-suspended in a small volume of buffer that keeps them from breaking open (lysing). It is important to make this suspension of cells homogeneous because cells trapped in clumps will be resistant to lysis reagents. Solution 1 contains RNase A; however, it cannot digest RNA until the cells are lysed in the next step.

13. **Check Solution 2. If precipitated, heat to 55° - 65°C for 5 minutes to dissolve. Be sure to cool to room temperature and mix before use.**
    What's happening: Solution 2 contains a detergent SDS that can precipitate if cooled. This precipitate is easy to re-suspend by heating. For this reason, always store this kit at room temperature (20-25°C).
14. **Add 250 µl Solution 2 to the cell suspension.**
    What's happening: Alkaline cell lysis. Solution 2 is very alkaline (pH 12) and contains the detergent SDS. Addition of Solution 2 causes the bacterial cells to lyse because the proteins in the cell membrane become denatured similar to when you cook an egg. All DNA becomes denatured to its single stranded form at this point. The bacterial chromosomal DNA is long and is attached to broken pieces of the cell membrane. Plasmid DNA is linked so it forms two attached circles. Like two links of a chain. All RNA is digested during this very short step because RNase A is active even in very alkaline conditions.
15. Close all tubes.
16. Gently invert the tubes 4-6 times to mix. **Do not vortex at this step.** Vortexing will reduce the quality of your plasmid prep. It causes chromosomal DNA contamination by breaking pieces of the bacteria's chromosomal DNA which will then co purify with the plasmid DNA.
17. **Add 350 µl Solution 3.**
    What's happening: Neutralization. Solution 3 contains potassium acetate and salt. The potassium acetate forms a precipitate when it interacts with SDS. At the same time denatured proteins co-precipitate with the SDS. Solution 3 neutralizes the alkaline pH to a more neutral pH 7. All DNA tries to re-nature. Plasmid can easily re-form to its double stranded form. Bacterial chromosomal DNA finds it difficult to re-nature because it has no reference point and homologous pieces of DNA may be blocked from finding each other by the cell debris present.
18. Close the tubes and gently invert 4-6 times to mix. **Do not vortex at this step.** Vortexing causes chromosomal DNA contamination.
19. Centrifuge for 1 minute. (10,000 x g minimum).
    What's happening: Dense cell debris is pelleted to the bottom of the tube. Chromosomal DNA is also pelleted along with the cell debris.
20. Remove the tubes from the centrifuge. There should be a clear non viscous supernatant on top of a large white pellet stuck to the sides of the tube. If the pellet is not firm but instead it is loose or gloppy, this is a clear indication you did not remove all the culture media when you originally pelleted the cells. You will need to start over in this case and be more careful to remove all the culture media.
21. Open the cap of as many spin filters as you have plasmid preps.
22. Transfer <u>all</u> of the clear liquid supernatant to a spin filter. (avoid the white precipitate). Decanting is the best method here. Just turn the tube so that you pour away from the hinge. The white pellet will stay stuck to the side of the tube. Close the lids of the spin filters.
23. Centrifuge 30-60 seconds. The liquid will flow through the white spin filter membrane leaving the plasmid DNA bound to the filter membrane.
    What's happening: The plasmid DNA now binds to the white silica membrane in the spin filter. Plasmid DNA binds due to the high salt conditions. Unwanted impurities such as digested RNA, and any other cell components that did not pellet are passed through the spin filter and end up in the flow through in the collection tube. This flow through is discarded.
24. (Precaution: Do not let the liquid in the spin filter collection tube come in contact with bleach.). Lift out the plastic filter basket from the collection tube, discard the liquid from the collection tube, and then replace the filter basket into the tube.
25. **Add 700 µl Solution 4** to the spin filter. Close the lid.
26. Centrifuge 30-60 seconds at 10,000 x g.
27. Discard flow through liquid from the collection tube, and centrifuge again for 1 minute.
    What's happening: Solution 4 washes the DNA that is bound to the spin filter. Solution 4 is about 50% ethanol. The ethanol keeps the plasmid DNA bound to the filter as impurities are washed away.

28. Being careful not to splash liquid on the filter basket, place spin filter basket in a new 2.0 ml collection tube (provided).
29. **Add 50 µl of Solution 5** or sterile water directly in the middle of the white spin filter membrane. Let stand for 1 minute.
    What's happening: Solution 5 is 10mM Tris. As it passes through the spin filter, the plasmid DNA is released (eluted) off the filter and it passes into the collection tube. The plasmid DNA is released because it will not stay bound to the spin filter when there is no salt present.
30. Centrifuge 1 minute at 10,000 x g.
31. Remove filter basket and close tube lid. Plasmid DNA in the collection tube is now ready to use for any application.

Thank you for choosing the Mo Bio UltraClean™ Standard Mini Plasmid Prep Kit

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

Version 02272007

Short Protocol (If already familiar with kit)

Note: All centrifuge steps are 10,000 x g. Remember to orient tubes with hinges pointing straight out from center of centrifuge rotor for all centrifuge steps.

Equipment required:

Microcentrifuge (10,000 x g), vortex, 80 well microcentrifuge tube rack (optional)
2 Pipettors: It makes things easier to have two pipettors: a P200 set to 50 µl and a P1000 set to 250 µl.

1. Pellet 2ml of overnight culture by centrifuging for 1 minute. Decant liquid media and spin again for 5 seconds. Remove the remaining liquid media with a small bore pipet tip.

2. **Resuspend cell pellets in 250µl of Solution 1**. Bump vortex until the mixture is completely homogeneous and no clumps exist.

3. **Add 250µl of Solution 2** and gently invert 4-6 times to mix. Do not vortex! This will cause shearing of the Genomic DNA.

4. **Add 350µl of Solution 3** and invert 4-6 times to mix. The resulting solution will be cloudy.

5. Centrifuge for 1 minute.

6. Transfer the supernatant to the spin filter unit. Avoid the white pellet. Decanting is the best method to transfer the supernatant.

7. Centrifuge 30 – 60 seconds. Discard the flow – through.

8. **Add 700µl of Solution 4 to the spin filter unit.**

9. Centrifuge for 30 – 60 seconds.

10. Discard the flow through. **Important**; centrifuge for 1 minute to remove the residual wash solution.

11. Transfer the spin filter basket to another 1.5 ml centrifuge tube. Be careful not to splash any Solution 4 on the spin filter!

12. **Add 50µl of Solution 5** or sterile water to the center of the spin filter membrane. Let stand at room temperature for 1 minute. Centrifuge for 1 minute.

13. Remove the spin filter basket, now the DNA is ready for any application.

Thank you for choosing Mo Bio Labs' UltraClean Standard Mini Plasmid Prep Kit.

Version 09012006

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

Hints and Troubleshooting Guide

Concentrating the DNA

Your final volume will be 50µl. If this is too dilute for your purposes, add 2 µl of 5M NaCl and mix. Then add 100 µl of 100% cold ethanol. Mix. Centrifuge at 10,000 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 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 30 seconds in a 2 ml tube (provided). Discard supernatant. Repeat until 5 ml of culture has been processed into one tube.

Low recovery

- **Two common causes of low yields:**

1. **The media was not completely removed**. In this case, the pellet that results from spinning, after Solution 3 has been added, is not a firm white pellet on the side of the tube. The pellet is loose and it tends to be difficult to remove any liquid cell lysate. If you see this, start the prep over and be very careful to follow the recommended way to remove the culture media below.

- **A good method to remove all the culture media:**
  This method requires two spins. Be careful to orient the tubes in the rotor the same way each time you spin them. Insert the tubes in the centrifuge rotor so that the cap hinge is pointed straight out away from the center of the rotor. The first spin (1 minute) will pellet the cells. The large volume of culture media can then be dumped into a waste receptacle (typically a bottle containing bleach) by inverting the tube. The cap should then be replaced and the tubes oriented in the rotor the same way. The second spin (5 seconds) will push all the residual media from the walls and cap to the bottom of the tube. If the tube was oriented the same as the original spin, the pellet will be very tight and the media will be easy to remove with a pipet tip. The best tips to use for this are the drawn out kind used to load sequencing gels. The small bore tip end makes removing very small volumes easier however; regular tips can also be used. The cells can now be resuspended in Solution 1 (see the notes below).

2. **The cell pellet was not completely resuspended.** In this case, a low yield results because clumps of cells do not lyse efficiently. There are a few good ways to resuspend the cells properly.

   A. Bump the tube on the vortex every 10 seconds during the 1 minute of vortexing it will take to resuspend the cells. Bumping means hold the tube on the vortex head for 10 seconds, take it off for 1 second then hold it on the vortex again. After 1 minute, hold the tube in a horizontal position up to a light and look at it. If you see any clumps of cells, keep bump vortexing until they are gone. It takes a minimum of 1 minute with the vortex at its highest speed to resuspend cells in the 50 µl volume. Do two tubes at a time when processing multiple preps.

   B. There is a paper in *Biotechniques*[1] that explains how cells can be resuspended in 5 seconds by scraping a tube across the holes of an 80 well microcentrifuge tube rack. The tube is held firmly in hand and the tube tip is run across the holes rapidly back and forth. It's easy and all you need is an 80 well rack. (They are great for storing tubes, most labs have them already).

1. Voo, K. S.; Jacobsen, B. M. *BioTechniques 24:240-243, February 1998*

If DNA floats out of well when loaded on a gel

Residual Solution 4 in is in the final sample. Prevent this by being careful not to transfer Solution 4 liquid onto the bottom of the spin filter basket. Ethanol precipitate to remove residues of Solution 4. See concentrating DNA above.

Other UltraClean™ Kits available from Mo Bio Laboratories, Inc.

Kit description	Cat. number
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:
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