

UltraClean™ 25 to 50 ml Plasmid Prep Kit

Catalog # 12700-20
20 preps

Instruction Manual

Introduction

Use this kit for isolating plasmid DNA from:

- A. 25ml of E. coli host strain cultures grown in high nutrient media such as TB DRY™ (*TB DRY™ is a single powder formulation of Terrific Broth. Just autoclave and use. Available through Mo Bio Laboratories, Inc.*), Super broth, and 2X YT. **OR,.....**
- B. 50ml of culture using low nutrient media such as LB broth.

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:

Vortex

Centrifuge capable of spinning 15ml tubes at a minimum of 2500-3500 x g and 50ml tubes at a minimum of 2500-3500 x g.

Note max. g force for 15ml tubes provided in this kit is 7,100 x g and for 50 ml tubes is 9,000 x g. **Swinging bucket rotor is recommended.**

Kit Contents: Reagents sufficient for 20 preps.

<u>Component</u>	<u>Amount</u>	<u>Description</u>
Solution 1:	10 ml	Cell suspension buffer: Tris, EDTA, RNase A
Solution 2:	18 ml	Cell lysis solution: SDS, and NaOH.
Solution 3:	52 ml	Binding buffer: Pot. Acetate/ Binding salt
Solution 4:	2x30 ml	Ethanol, Tris, NaCl. Caution: Flammable
Solution 5:	22 ml	Elution buffer containing 10 mM Tris-HCl.
Spin Filters	20	Spin filter baskets.
15 ml tubes	60	Centrifuge tubes.
50 ml tubes	20	Centrifuge tubes.

Kit Storage

Room temperature for 1 year.

Do not let Solution 3 come into contact with bleach or other oxidizers.

Solution 4 is Flammable. Use caution.

Detailed Protocol

(If you are already familiar with this kit, see the short protocol)

Please wear gloves at all times

1. **In this kit, Solution 4 already contains ethanol.**
2. In a 50ml tube, centrifuge up to 25ml of an overnight culture of high nutrient media such as Terrific Broth or TB Dry™ or up to 50ml of LB Broth culture for 10 minutes at 2500-3500 x g. For maximum yields, the supernatant should be clear and the bacteria should form a tight pellet. If the supernatant is not clear, centrifuge longer or at a higher g force.
What's happening: The bacterial cells are being forced to the bottom of the tube.
3. Discard the supernatant. **IMPORTANT:** Drain tube of **all** liquid. Placing the tube upside down on an absorbent paper towel for a few minutes will drain all the liquid.
What's happening: The cells have been pelleted and are now separated from the culture growth medium.
4. **Add 0.4ml Solution 1** and re-suspend the bacterial pellet by vortexing until homogeneous. (Be sure no clumps exist). Re-suspend 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 re-suspend cells.
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.
5. **Check Solution 2 before use.** If a precipitate has formed, heat to dissolve. Cool to room temperature ~20-25°C before using.
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).
6. **Add 0.8ml Solution 2**, gently swirl to mix and then let stand for 30 seconds.
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.
7. **Add 2.4ml Solution 3** and invert twice to mix.
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.
8. Transfer to a 15ml tube (provided).
9. Centrifuge at 2500-3500 x g for 10-15 minutes. 3500 x g for 10 minutes is more efficient, but if your centrifuge cannot attain this force, 2500x g for 15 minutes is sufficient. If your centrifuge can spin a 50 ml tube at 3500 x g, you do not need to transfer to the 15 ml tube. In that case, just spin the 50 ml tube at 3500 x g for 10 minutes.

What's happening: Dense cell debris are pelleted to the bottom of the tube. Chromosomal DNA is also pelleted along with the cell debris.

10. Place a spin filter in a 15ml centrifuge tube (provided).
 11. Transfer the liquid lysate supernatant to a spin filter. (Avoid the white precipitate when transferring the supernatant). The best way to do this is to keep the white pellet on the up side of the tube as you pour the liquid into the spin filter. If the white pellet does not appear tight enough to pour out the liquid, it may be necessary to centrifuge again. Sometimes there is a floating white material. Try to avoid pouring this into the spin filter. It can be held back with a pipet if necessary.
 12. Close the lid and centrifuge for 3 minutes at 2500-3500 x g.
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.
 13. **Warning:** Do not expose the liquid that flows through the spin filter and into the collection tube to bleach. Remove the filter unit, discard the liquid contents from the collection tube in a suitable container and then replace the filter unit in the tube.
 14. **Add 3ml Solution 4** to the spin filter.
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.
 15. Centrifuge 3 minutes at 2500-3500 x g.
 16. Carefully place spin filter unit in a new 15ml tube without splashing any liquid on the spin filter as it is removed. (Optionally, you may want to remove the spin filter unit and discard to liquid from the collection tube. Replace the spin filter unit in the same collection tube. Centrifuge again for 3 minutes at 2500-3500 x g. then transfer the spin filter unit to a new 15 ml tube. This will insure that no residual Solution 4 will be left on the spin filter.)
 17. **Add 1ml Solution 5** to the middle of the spin filter.
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.
 18. Centrifuge for 3 minutes at 2500-3500 x g.
 19. Remove filter unit and close tube lid.
- Plasmid DNA is now ready to use for any application.

Thank you for choosing the UltraClean™ Plasmid Prep Kit.

Version 03222005

Short Protocol

Please wear gloves at all times

1. Centrifuge at 2500-3500 x g for 10 minutes: in a 50 ml tube provided either 25 ml of high nutrient culture or 50 ml of LB culture.
2. Discard supernatant and drain tube inverted on a paper towel for 2 minutes.
3. **Add 0.4ml Solution 1**
4. Re-suspend the bacterial pellet by bump vortexing until homogeneous.
5. **Check Solution 2 before use.** If a precipitate has formed, heat to dissolve. Cool to room temperature ~20-25°C before using.
6. **Add 0.8ml Solution 2**, gently swirl to mix and then let stand for 30 seconds.
7. **Add 2.4ml Solution 3** and invert twice to mix.
8. Transfer to a 15ml tube (provided).
9. Centrifuge at 2500-3500 x g for 10 minutes.
10. Place a spin filter in a 15ml centrifuge tube (provided).
11. Transfer the lysate supernatant to a spin filter. (Avoid the white precipitate)
12. Centrifuge for 3 minutes at 2500-3500 x g. **Warning:** Do not expose the liquid flow through to bleach.
13. Discard the flow through then replace the filter unit in the tube.
14. **Add 3ml Solution 4** to the spin filter.
15. Centrifuge 3 minutes at 2500-3500 x g.
16. Carefully place spin filter unit in a new 15ml tube without splashing any liquid on the spin filter
17. **Add 1ml Solution 5** to the middle of the spin filter.
18. Centrifuge for 3 minutes at 2500-3500 x g.
19. Remove filter unit and close tube lid.

Plasmid DNA is now ready to use for any application.

Thank you for choosing the UltraClean™ Plasmid Prep Kit.

Version 03222005

Hints and Troubleshooting Guide

Concentrating the DNA: Your final volume will be 1 ml. DNA is in 10 mM Tris. If this is too dilute for your purposes, ethanol precipitate the DNA to concentrate it. Add 1/10 vol. salt (3 M Potassium acetate) and 2 volumes 100 % cold ethanol. Centrifuge 10 min. Discard supernatant. Note position of pellet. Drain tube on a paper towel for 5 minutes. Re-suspend pellet in desired volume of Solution 5.

Amount of culture to process: This kit is designed for up to 25ml of **high nutrient media**. If you are using LB or other lower nutrient media, use up to 50ml of culture. To get higher yields use TB DRY High nutrient media. (A one powder form of Terrific broth manufactured by Mo Bio Laboratories, Inc.).

White pellet after Solution 3 neutralizing step is loose: If you do not get a tight white pellet after adding Solution 3 and centrifuging but rather a loose pellet or flocculent solution, you may have left too much residual culture media in the tube after spinning down the cells. You will need to start over and be careful to remove all traces of liquid culture media after pelleting the cells.

DNA floats out of well when loaded on a gel: You may have inadvertently transferred some residual Solution 4 into the final sample. Prevent this by being careful not to transfer Solution 4 flow through onto the bottom of the spin filter basket prior to the elution step with Solution 5. If you suspect you have residual Solution 4 in your sample, ethanol precipitation is the best way to remove residues of Solution 4. See "Concentrating the DNA" above.

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

email: orders@mobio.com

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/