

# UltraClean™ 250 to 500ml Plasmid Prep Kit

Catalog # 12600-20  
20 preps  
**Instruction Manual**

## Introduction

Use this kit for isolating plasmids from up to 500ml of E. coli host strain cultures grown in LB media or 250ml of high nutrient media such as TB DRY\*.

## 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](http://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.**

Vortex Centrifuge capable of spinning 250 - 500ml bottles at a minimum of 2500 x g and 50ml tubes at 2500 x g. **Note maximum g force rating of 50 ml tubes is 9,000 x g. Do not exceed 6,000 x g in a 50 ml tube filled with 50 ml of culture in the tubes provided.**

**A swinging bucket rotor is recommended.**

**Kit Contents:** Sufficient reagents for 20 preps.

<u>Component</u>	<u>Amount</u>	<u>Description</u>
Solution 1:	105 ml	Cell suspension buffer: Tris, EDTA, RNase A
Solution 2:	210 ml	Cell lysis solution: SDS, and NaOH.
Solution 3:	630 ml	Binding buffer: Pot. acetate/ binding salt
Solution 4:	210 ml	Tris, NaCl. <b>(before first use: add 210ml 100% ETOH and remove label from cap)</b>
Solution 5:	88 ml	Elution buffer containing 10 mM Tris-HCl.
Spin Filters	20	Spin filter basket in a 50ml tube.
50ml tubes	40	Centrifuge tubes.

## Kit Storage

Room temperature for 1 year.

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

- TB DRY™ is a single powder formulation of Terrific Broth. Just autoclave and use. (Available through Mo Bio Laboratories, Inc.)

## Detailed Protocol

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

**Please wear gloves at all times.**

1. **Before first use, prepare solution 4. See Kit Contents section.**
2. Centrifuge up to 250ml of an overnight culture of high nutrient media such as Terrific Broth or TB Dry™ or up to 500ml of LB Broth culture for 15 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. Do not exceed 6,000 x g in a 50 ml tube filled with 50 ml of culture in the tubes provided.  
*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 5ml 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 10ml 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 30ml 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 50ml 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. *What's happening: Dense cell debris is pelleted to the bottom of the tube. Chromosomal DNA is also pelleted along with the cell debris.*

10. Transfer 20ml of 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.
  11. Close the lid and centrifuge for 5 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.*
  12. **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.
  13. Add remaining lysate to spin filter and repeat step 10-11.
  14. **Add 20ml Solution 4 (Ethanol already added)** 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 5 minutes at 2500-3500 x g.
  16. Remove spin filter, and discard liquid from tube. Replace spin filter, and spin again for 5 min.
  17. Carefully place spin filter unit in a new 50ml tube without splashing any liquid on the spin filter as it is removed
  18. **Add 4ml Solution 5** to the middle of the spin filter. (Note: Lower volumes can be used for this DNA elution step. This will concentrate the DNA. To elute in lower volumes, please see Hints and Troubleshooting guide "Eluting in Lower Volumes").  
*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.*
  19. Centrifuge for 5 minutes at 2500-3500 x g.
  20. 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.**

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## Short Protocol

(Before **first** use, prepare Solution 4. See Kit Contents section)

**Please wear gloves at all times**

**For centrifugal forces needed, see Equipment required section.**

1. Centrifuge 500ml of LB culture or 250ml high nutrient broth (TB DRY. Terrific broth, 2X YT) culture for 15 min. at 2500 x g.
  2. Discard the supernatant. Be sure **all** liquid is removed.
  3. **Add 5ml Solution 1** and resuspend the bacterial pellet by vortexing until homogeneous (be sure no clumps exist).
  4. **(Check Solution 2 before use.** If a precipitate has formed, heat to dissolve).
  5. **Add 10ml Solution 2** and swirl gently to mix.
  6. Incubate at room temperature for 30 seconds.
  7. **Add 30ml Solution 3** and invert twice to mix.
  8. Transfer to a 50ml tube and centrifuge for 10 min. at 2500 x g.
  9. Transfer 20ml of the lysate supernatant to a spin filter. (Avoid the white precipitate when transferring the supernatant).
  10. Centrifuge 5 minutes at >2500 x g.
  11. **Warning:** Do not let this liquid contact bleach. Remove filter unit, discard the liquid contents from tube and then replace the filter unit in the tube.
  12. Add the remaining lysate to the spin filter and repeat step 9.
  13. Discard the liquid and replace the spin filter.
  14. **Add 20ml Solution 4 (ethanol already added)** to the spin filter.
  15. Centrifuge 5 minutes at 2500 x g
  16. Discard liquid from tube, replace spin filter and spin again 5 min.
  17. Carefully place spin filter unit in a new 50ml tube without splashing any liquid on the spin filter as it is removed.
  18. **Add 4ml Solution 5** or sterile water \*Note: Use sterile water to elute if you plan to use the plasmid for transfections. (Note: Lower volumes can be used for this DNA elution step. This will concentrate the DNA. To elute in lower volumes, please see Hints and Troubleshooting guide "Eluting in Lower Volumes").
  19. Centrifuge 5 minutes at 2500 x g.
  20. 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.**

## Hints and Troubleshooting Guide

### Concentrating the DNA

Your final volume will be 4 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 Sodium acetate) and 2 volumes 100 % cold ethanol.

### Eluting in Lower Volumes

DNA can be recovered from the spin filter in a lower volume than 4 ml. This will help concentrate DNA automatically. Instead of adding 4 ml of Solution 5 as directed in the protocol follow this procedure: Heat Solution 5 to 65 C. Add 1 ml directly to center of spin filter membrane. Incubate at room temperature for 1 minute. Centrifuge 5 minutes at 2,500 x g. Remove filter unit and close tube lid. Plasmid DNA is now ready to use for any application. If DNA is intended for transfection, use sterile water in place of Solution 5.

**Amount of culture to process:** This kit is designed for up to 250ml of high nutrient media and 500ml of LB culture. To get higher yields use TB DRY™ High nutrient media. (A single powder form of Terrific broth).

**Incomplete Lysis:** If you do not get a white pellet after adding Solution 3 and centrifuging but rather a flocculent solution, you may have left too much media in the tube at step 2. Repeat. Be careful during step 2 to remove all traces of liquid. Try using 200ml of high nutrient culture, or 450ml of LB.

**Low recovery:** Little plasmid produced by the host. Increase yields with high nutrient media such as TB DRY (a single powder formulation of Terrific Broth). Other causes are too much residual media was left in the tube at step 2. Or overloading the system with too many cells. Repeat. Be careful during step 2 to remove all traces of liquid. Try using 200ml of high nutrient culture, or 450ml of LB.

**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 in step 15 not to transfer liquid onto the bottom of the spin filter basket. Ethanol precipitation is the best way to remove residues of Solution 4.

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

<u>Kit description</u>	<u>Cat. number</u>
<b>Plasmid Prep Kits</b>	
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
<b>Endotoxin-Free Plasmid Prep Kits</b>	
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
<b>DNA Purification Kits</b>	
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
<b>DNA Isolation Kits</b>	
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
<b>RNA Isolation Kits</b>	
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
<b>Growth Media</b>	
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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