

UltraClean™ Endotoxin-Free Ethanol Precipitation Kit

Catalog # 12616
Instruction Manual

Introduction

This kit is designed to provide Endotoxin-Free ethanol precipitation of DNA at levels $<0.1\text{EU}/\mu\text{g}$. Endotoxin-Free DNA solutions may be concentrated with this kit; **however this kit does not remove endotoxins**. To remove endotoxin from purified DNA, we recommend the Mo Bio UltraClean Endotoxin Removal Kit (Catalog # 12615). The concentrated Plasmid DNA obtained after precipitation is suitable for transfection, injection, automated sequencing, and most other downstream applications. Endotoxins, also known as lipopolysaccharides or LPS, are cell-membrane components of Gram-negative bacteria such as *E. coli*. Endotoxins are released during the lysis step of plasmid purification and significantly reduce transfection efficiencies in endotoxin-sensitive cell lines. Because endotoxins represent a non-controllable variable in transfection experiments, their presence can make results difficult to compare and interpret. This kit contains all certified Endotoxin-Free reagents necessary for DNA concentration.

Recommendations when using this kit

- ◆ **This kit is only intended to concentrate DNA that is already Endotoxin-Free.**
- ◆ Use only for DNA solutions containing less than 100mM salt. This kit can be used for high salt solutions after salts have been diluted to 100mM with water.
- ◆ Use only certified Endotoxin-Free pipet tips and centrifuge tubes with this kit.
- ◆ Use sterile technique for all steps. The use of a laminar flow hood is recommended.
- ◆ **This kit is for research purposes only. Not for diagnostic or clinical 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.

Equipment required:

Microcentrifuge (15,000 x g)

Kit Contents:

<u>Component</u>	<u>Amount</u>
Endotoxin-Free 5M Sodium Chloride	10 ml
Endotoxin-Free 100% Ethanol	2x30 ml
Endotoxin-Free Water	10 ml
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Kit Storage

Room temperature for 1 year

WARNING: 100% Ethanol is very flammable. Avoid open flames and high heat.

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

Protocol

Please wear gloves at all times

1. Add 1/10 volume of Endotoxin-Free 5M Sodium Chloride.
2. Mix by pipetting.
3. Add 2 volumes of Endotoxin-Free Ethanol.
4. Mix by inverting tube several times.
5. Incubate on ice for 30 minutes.
6. Centrifuge 15 minutes at 15,000 x g.
7. Carefully remove the supernatant by pipetting and discard. Do not disturb the small DNA pellet.
8. Wash pellet carefully with 1 ml 70% ethanol. (70% Endotoxin-Free Ethanol can be made from the 100% ethanol and water supplied in this kit by adding 0.7 ml of 100% Endotoxin-Free ethanol to 0.3 ml of supplied Endotoxin-Free Water).
9. Invert tube several times gently.
10. Centrifuge 5 minutes at 15,000 x g.
11. Discard supernatant and leave tube inverted upside down to drain for 5 minutes on a clean tissue.
12. Dry pellet. This can be done by allowing tube to sit in a Class 100 laminar flow hood for 15-30 minutes.
13. When the pellet is dry, resuspend it in the Endotoxin-Free Water supplied with the kit.

You are now ready for applications requiring Endotoxin-Free DNA.

Version 03222005

Thank you for choosing the UltraClean Endotoxin-Free Ethanol Precipitation Kit

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

<u>Kit description</u>	<u>Cat. number</u>
Endotoxin Removal Kit	12615
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
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
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
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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For the distributor nearest you, go to our web site at www.mobio.com/distributors/