



UltraClean™ Endotoxin Removal Kit

Catalog # 12615

Instruction Manual

Introduction

This kit is designed to remove endotoxins from low salt solutions. Solutions containing high salt concentrations should be diluted to 100mM or less before using this kit. DNA solutions may be concentrated with the Endotoxin free Ethanol Precipitation reagents included in this kit. Once plasmid DNA has had endotoxins removed with this kit, endotoxin levels are <0.1EU/μg. The Plasmid DNA obtained is then suitable for transfection, injection, automated sequencing, and most other downstream applications.

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 (15,000 x *g*)
65°C water bath, Vortex

Kit Contents:

<u>Component</u>	<u>Amount</u>
Endotoxin Removal Reagent	25 ml
Endotoxin free 100% Ethanol	2x30 ml
Endotoxin free Water	10 ml
Endotoxin free 5M Sodium Chloride	10 ml
Instruction Manual	

Kit Storage

Room temperature for 6 months

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

Version: 02082008

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



Protocol

- ◆ Use only for 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.

Please wear gloves at all times

1. **Add 1/10 volume of Endotoxin Removal Reagent** to your sample. (Sample cannot contain more than 100mM salt). **Note:** The Endotoxin Removal Reagent is a viscous solution. To pipette this reagent draw up and dispense slowly.
2. **Vortex** at highest speed (vigorously) until homogeneous. Solution will become turbid and red in color after the addition of Endotoxin Removal Reagent.
3. Incubate on ice or at -20°C for 5 minutes.
4. Heat at 65°C for 10 minutes.
5. **Centrifuge for 5 minutes at 15,000 x g.** After centrifugation, the top aqueous phase will be clear and the bottom phase will be red and viscous.
6. Collect the top aqueous phase with a certified endotoxin free pipet tip. Be sure to avoid the red bottom phase as this contains concentrated endotoxin.
7. Transfer the aqueous phase to a new tube.
8. **Optional: Repeat the extraction procedure one more time as described in step 1-6. The more times you extract, the cleaner the sample becomes. For plasmid preps, a total of 2 extractions are typically required.**
9. **Centrifuge for 5 minutes at 15000 x g. Check for a small red pellet at the bottom of the tube. If present, transfer the top phase without disturbing the red pellet to another tube.**

Endotoxin Free Ethanol Precipitation of DNA

- ◆ To concentrate DNA samples.
 - ◆ Use sterile technique for all steps.
 - ◆ Use only certified endotoxin free and sterile pipet tips and centrifuge tubes for the following procedure.
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 discard supernatant.** Do not disturb 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).
 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 pellet is dry, resuspend in Endotoxin free Water supplied.

DNA is now ready for applications requiring endotoxin free DNA.

Thank you for choosing the UltraClean™ Endotoxin Removal Kit.

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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

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Technical information:

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Ordering Information

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