MiraCLEAN® Endotoxin Removal Kit

Protocol for MIR 5900, 5910

Quick Reference Protocol, SDS and Certificate of Analysis available at mirusbio.com/5900

INTRODUCTION

Many laboratory applications require endotoxin-free preparations of plasmid DNA. However, the outer membrane of *E. coli*, a Gram-negative eubacteria commonly used for plasmid production, contains lipopolysaccharides (i.e. endotoxin) which cause inflammatory reactions, fever and endotoxic shock *in vivo* and decrease transfection efficiencies *in vitro*. The MiraCLEAN® Endotoxin Removal Kit provides a convenient method for the removal of bacterial endotoxins from DNA for both *in vivo* and *in vitro* applications.

The MiraCLEAN® Endotoxin Removal Kit is based on a rapid phase extraction that efficiently removes endotoxin contamination from DNA. The proprietary EndoGO Extraction Reagent allows better visualization of the phase separation interface which facilitates enhanced recovery of nucleic acid. One MiraCLEAN® Endotoxin Removal Kit contains sufficient reagent to perform three endotoxin extraction rounds on approximately 10 mg (MIR 5910) or 100 mg (MIR 5900) of DNA.

NOTE: This kit has not been tested for endotoxin removal in RNA or protein samples.

For more information on the MiraCLEAN® Endotoxin Removal Kit, please visit the product page at www.mirusbio.com/5900.

SPECIFICATIONS

Storage	Store EndoGO Extraction Reagent at 4°C. ***Before each use***, warm to room temperature and vortex gently. Store MiraCLEAN® Buffer at 4°C.
Stability/ Guarantee	1 year from the date of purchase, when properly stored and handled.

MATERIALS

Materials supplied

The MiraCLEAN® Endotoxin Removal Kit is supplied in one of the following formats:

Product No.	EndoGO Extraction Reagent Volume	MiraCLEAN® Buffer Volume	Sufficient material to perform the following:
MIR 5910	2.2 ml	2 ml	Three endotoxin extraction rounds on ~10 mg of DNA
MIR 5900	22 ml	20 ml	Three endotoxin extraction rounds on ~100 mg of DNA

CAUTION: Standard safe laboratory practices should be maintained when using all chemical transfection reagents. ***Please refer to product SDS for full safety precautions.***

Materials required, but not supplied

- DNA sample at 0.5 - 1 mg/ml in 1X TE, pH 7.4-8.0 (10mM Tris, 1mM EDTA)
- Absolute (100%) Ethanol at -20°C
- Sterile 1.5 ml microcentrifuge tubes
- 50°C water bath or heat block
- Microcentrifuge
- Small container of ice for incubations

For Research Use Only.

ENDOTOXIN REMOVAL PROTOCOL

The following procedure describes how to remove endotoxin contaminants from plasmid DNA using the MiraCLEAN® Endotoxin Removal Kit. Two extraction rounds with the MiraCLEAN® Kit are typically sufficient to reduce endotoxin contamination in plasmids from 50,000 EU/ml to <30 EU/ml, which is compatible with *in vivo* and *in vitro* applications. **NOTE:** A variety of commercial kits are available to detect and/or quantify the presence of endotoxin in samples. We recommend the QCL-1000 Chromogenic LAL Testing Kit (Lonza, 50-647U or 50-648U) to assess endotoxin levels.

Procedure

A. Before you begin

1. Set a water bath or heat block to 50°C and verify the temperature with a thermometer.
2. Prepare an ice bath.
3. Warm the EndoGO Extraction Reagent to room temperature and vortex to mix. NOTE: The MiraCLEAN® Buffer does not need to be warmed prior to use.

Warm EndoGO Extraction Reagent to room temperature and vortex before each use.

B. MiraCLEAN® Endotoxin Extraction Procedure

1. Dilute the DNA sample to 0.5 – 1.0 mg/ml using TE buffer. **NOTE:** If TE is not available, dilute in water, MOPS buffer, low salt-Tris buffers, or other comparable buffers.
2. Add 0.1 volumes of MiraCLEAN® Buffer to the DNA and vortex to mix.
3. If necessary, distribute the DNA sample mixture into several microcentrifuge tubes, ensuring a maximum volume of 1.2 ml per tube. **NOTE:** Phase distinction in the subsequent steps is difficult to detect with volumes <50 µl. Ensure that each sample is 50 µl - 1.2 ml in volume.
4. Incubate the samples in the ice bath for at least 5 minutes.
5. Vortex the EndoGO Reagent, then add 0.03 volumes directly to the DNA sample within each tube. **NOTE:** EndoGO Extraction Reagent is highly viscous and difficult to pipet in small quantities. For best results, snip off the end of the pipette tip and slowly remove desired quantity. For this step, extreme accuracy is not required.
6. Briefly vortex the samples and incubate on ice for 5 minutes. Vortex intermittently (at least 2 times) during the incubation.
7. Incubate the samples at 50°C for ≥5 minutes. Incubation times up to 30 minutes may be required for samples containing high levels of endotoxin. **NOTE:** A 50ºC temperature is required for complete phase separation. Lower temperatures do not separate phases as efficiently which can lead to unsuccessful endotoxin removal.
8. Chill samples on ice for 3-5 minutes prior to centrifugation.
9. Centrifuge the tubes at room temperature for 1 minute at a minimum of 14,000 x g. **NOTE**: If complete phase separation does not occur, centrifuge for an additional 10 minutes.
10. Gently remove tubes from the centrifuge. Using a pipette and standard tip, carefully transfer the colorless upper aqueous phase containing the DNA to a new tube and place it in the ice bath. **NOTE:** Remove the upper aqueous phase slowly to avoid collapse of the phase interface. The lower pink phase contains the extracted endotoxin.

A 50°C incubation time of up to 30 minutes may be required for samples containing high levels of endotoxin.

11. Repeat steps B5 - B10 as needed. The number of extraction rounds required depends both on the quality and quantity of sample you wish to obtain. Additional extraction rounds may result in better purity but lower yield. The average loss of DNA per extraction round is 5-10%. Two rounds of extraction are recommended for samples containing moderate endotoxin contamination (e.g. <50,000 EU/mg) with an additional round of extraction for samples with significant endotoxin contamination.

C. Ethanol purification of final DNA sample

1. Precipitate the DNA by adding 2-2.5 volumes of ice cold 100% ethanol to the tube.
2. Mix well and incubate at ≤ -20ºC for at least 30 minutes.
3. Centrifuge at max speed (>14,000 x g) in a refrigerated microcentrifuge for 20 minutes to pellet the DNA. Gently remove the ethanol with a micropipetter. Do not disturb the pellet.
4. Wash the pellet once with up to 500 µl room temperature 70% ethanol. Centrifuge at max speed in a refrigerated microcentrifuge for 15-20 minutes.
5. Remove all traces of ethanol with a micropipetter. DO NOT allow the samples to air dry for longer than 5 minutes as the pellet may become difficult to resuspend.
6. Resuspend pellet in desired volume of buffer of choice.
7. Store the purified DNA on ice for immediate use or at -20°C for long-term storage.

TROUBLESHOOTING GUIDE

Unsatisfactory Endotoxin Removal Reaction	
Problem	**Solution**
Suboptimal Phase Separation	A 50ºC water bath is required for complete phase separation. Using a lower temperature does not separate phases as efficiently which can lead to unsuccessful endotoxin removal.
	Samples that are not placed on ice immediately after heating will not achieve complete phase separation.
	Increasing water bath incubation time up to 30 minutes (step B7) and centrifuge spin time up to 10 minutes (step B9) may provide a more successful extraction of the endotoxin in the sample. Do not shorten recommended incubation or centrifuge time points.
	If phases do not separate clearly after increasing the duration of the 50°C incubation or post-incubation centrifugation, perform a phenol:chloroform extraction followed by an ethanol precipitation to recover DNA then repeat the MiraCLEAN® Endotoxin Removal steps.
Low DNA Yield	If final DNA concentration is lower than expected, increase initial DNA sample volume per tube (concentration should not exceed 1 mg/ml) and ensure maximal recovery of the aqueous phase during each round of extraction. With proper pipetting techniques, expected loss of DNA per round of extraction is 5-10%.

RELATED PRODUCTS

- Ingenio® Electroporation Solution and Kits
- *Trans*IT-X2® Dynamic Delivery System
- *Trans*IT®-2020 Transfection Reagent
- *Trans*IT®-LT1 Transfection Reagent
- *Trans*IT® Cell Line Specific Transfection Reagents and Kits
- CHOgro® Expression System
- *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kits

For details on the above mentioned products, visit www.mirusbio.com

Reagent Agent®

Reagent Agent® is an online tool designed to help determine the best solution for nucleic acid delivery based on in-house data, customer feedback and citations.

Learn more at:
www.mirusbio.com/ra

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