MiraCLEAN® Endotoxin Removal Kit

Quick Reference Protocol

Instructions for MIR 5900, 5910

Full protocol, SDS and Certificate of Analysis available at 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.
Product Guarantee	1 year from the date of purchase, when properly stored and handled.
Kit Sizes and Usage	MIR 5900 is sufficient for 3 endotoxin extraction rounds on ~10 mg of DNA. MIR 5910 is sufficient for 30 endotoxin extraction rounds on ~100 mg of DNA.

MiraCLEAN® Endotoxin Removal Protocol

Full protocol and additional documentation available at ***mirusbio.com/5900***

The outer membrane of *E. coli*, a Gram-negative eubacteria commonly used for plasmid production, contains endotoxins which cause inflammatory reactions, fever and endotoxic shock *in vivo* and decrease transfection efficiencies *in vitro*. The following procedure describes how to remove endotoxin contaminants from plasmid DNA using the MiraCLEAN® Endotoxin Removal Kit.

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.

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 buffer or other comparable buffer.
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 final volume between 50 µl - 1.2 ml per tube.
4. Incubate the samples in the ice bath for at least 5 minutes.
5. Vortex the EndoGO Reagent and add 0.03 volumes directly to the DNA sample in 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 the desired volume.
6. Briefly vortex the samples and incubate on ice for 5 minutes. Vortex at least twice during the incubation.
7. Incubate samples at 50°C for a minimum of 5 minutes. Incubations up to 30 minutes may be required for samples containing high levels of endotoxin. **NOTE:** A 50°C temperature is required for complete phase separation.
8. After the incubation, chill samples on ice for 3 - 5 minutes prior to centrifugation.
9. Centrifuge the samples 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 samples 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.
11. Repeat steps B5 - B10 as needed. The number of required extraction rounds will depend upon the desired quality and quantity of the final sample. Additional extraction rounds may result in better purity but lower yield, as the average DNA loss per extraction round is 5-10%. Two extraction rounds are recommended for samples containing moderate endotoxin contamination. An additional extraction round is recommended for samples with significant endotoxin contamination.

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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 ≤ -20°C 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 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.

Determining Endotoxin Levels

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. Two extraction rounds with the MiraCLEAN® Endotoxin Removal 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.

For Research Use Only

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