MiraCLEAN® Endotoxin Removal Kit

Product Name	Quantity	Product No.
MiraCLEAN® Endotoxin Removal Kit	For 10 mg DNA	MIR 5910
	For 100 mg DNA	MIR 5900

1.0 DESCRIPTION

1.1 General Information

Many molecular biology laboratory applications require endotoxin-free preparations of plasmid DNA and high molecular weight genomic DNA. The MiraCLEAN® Endotoxin Removal Kit provides a convenient and improved method for the removal of bacterial endotoxins from DNA for both *in vivo* and *in vitro* applications. *E. coli*, a Gram-negative eubacteria, is the common host for plasmid production. The outer leaflet of the outer membrane contains lipopolysaccharides (endotoxin) which can cause inflammatory reactions, fever and endotoxic shock *in vivo*. Endotoxin in plasmid preparations is also known to decrease transfection efficiencies *in vitro* (Osborn and Schindler, *Biotechniques*, 1995). The MiraCLEAN® Endotoxin Removal Kit is based on a rapid phase extraction that efficiently and conveniently removes endotoxin contamination from DNA. The proprietary pink-colored EndoGO Extraction Reagent allows better visualization of the interface thereby facilitating a phase separation and increasing the recovery of nucleic acid. One MIR 5910 Kit contains 2.2 ml of EndoGO Extraction Reagent and 2.0 ml of MiraCLEAN® Buffer which is sufficient to perform three endotoxin extraction rounds on at least 10 mg of DNA.

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

1.2 Specifications

Storage: Store EndoGO Extraction Reagent at 4º C (warm to room temperature and vortex before use)
Store MiraCLEAN® Buffer at 4ºC

Stability: 6 months when stored properly

Performance: Each batch of MiraCLEAN is tested for efficiency of endotoxin removal from contaminated plasmid DNA and must yield DNA purities of < 30 EU/mg

1.3 Required Equipment and Reagents

0.5-1 mg/ml DNA sample in 1X TE, pH 7.4-8.0 (10mM Tris, 1mM EDTA)
Absolute Ethanol (-20°C)
1.5 ml microcentrifuge tubes
50ºC water bath
Microcentrifuge
Ice

2.0 PROCEDURE

A. Set a water bath to 50°C. Prepare an ice bath.

B. Warm the EndoGO Extraction Reagent to room temperature and vortex before use in step G.

C. Dilute the DNA sample to 0.5 - 1.0 mg/ml using TE buffer.
NOTE: If TE is not available, dilute the DNA sample in water, MOPS buffer, low salt-Tris buffers, or other comparable buffers.

D. Add 0.1 volumes of MiraCLEAN® Buffer to the DNA then vortex to mix.

E. If necessary, aliquot the DNA samples into microcentrifuge tubes. For larger samples, dispense the DNA into a series of microcentrifuge tubes, with no more than 1.2 ml of DNA per tube.
NOTE: Using small aliquots (<50 ul) makes it difficult to distinguish phases during the phase extraction process, especially if the DNA sample is extremely contaminated with endotoxin. Ensure that each sample is at least 50 ul in volume.

F. Incubate the samples in the ice bath for at least 5 minutes.

G. Vortex, then add 0.03 volumes of the EndoGO Extraction Reagent before use to each tube. Because of its viscosity and tendency to stick to plastic, the EndoGO Extraction Reagent should be added directly to the DNA sample in the tube.
NOTE: EndoGO Extraction Reagent is 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.

H. Briefly vortex the samples and incubate them in the ice bath for 5 minutes with intermittent vortexing (at least 2 times).

I. Incubate the samples in the 50°C water bath for 5 minutes.
NOTE: A 50ºC water bath is required for complete separation of phases. Using a lower temperature does not separate phases as efficiently which can lead to unsuccessful endotoxin removal.

J. Using a standard bench top microcentrifuge, centrifuge the tubes at room temperature for approximately 20 seconds at 14000 x g or faster.

K. Gently remove tubes from the centrifuge. Carefully tilt the tube and transfer the upper colorless aqueous phase (containing the DNA), using a pipette and a standard tip, to a new tube and place it in the ice bath. Remove the upper aqueous phase slowly to avoid collapse of the interface between the two phases. The lower pink phase contains the extracted endotoxin.

L. Repeat steps G through K as needed. The number of extraction rounds required depends both on the quality and quantity of sample you wish to obtain. An extra round of extraction may result in better purity but slightly less yield. The average loss of DNA per extraction round is 5-10%. Two or three rounds of treatment are sufficient to completely remove (or reduce to undetectable levels) endotoxin from contaminated plasmid DNA preparations. Two rounds of extraction are recommended for samples expected to have moderate endotoxin contamination with an additional round of extraction for samples suspected to have significant endotoxin contamination (ie. alkaline lysis plasmid DNA).

M. Precipitate the DNA with 2 volumes of cold 100% ethanol. Incubate at -20ºC or colder for at least 30 minutes. Centrifuge at 14000 x g at 4ºC for 20 minutes. Remove supernatant and wash pellet with 70% ethanol. Centrifuge at 14000 x g at 4ºC for 20 minutes. Remove supernatant, and resuspend pellet in desired volume of buffer of choice.

NOTE: A variety of commercial kits are available to detect and/or quantify the presence of endotoxin in samples. We recommend BioWhittaker's QCL-1000® Chromogenic LAL Testing Kit (cat. # 50-647U or #50-648U) to assess endotoxin levels. The endotoxin level detected in DNA samples treated with the MiraCLEAN® Kit is less than 30 EU/mg, which is compatible with a variety of *in vivo* and *in vitro* applications.

3.0 TROUBLESHOOTING

Suboptimal Phase Separation

- If phrases do not separate, perform a phenol:chloroform extraction followed by an ethanol precipitation to recover DNA then repeat the MiraCLEAN® Endotoxin Removal steps.
- 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 the incubation times up to 10 minutes for centrifuge spin time and 30 minutes for water bath incubation time may provide a more successful extraction of the endotoxin in the sample. Do not shorten recommended incubation or centrifuge time points.

Low DNA Yield

- With proper pipetting techniques, expected loss of DNA per round of extraction is 5-10%.
- If final DNA concentration is lower than expected, increase initial DNA sample volume per tube (concentration should not exceed 1 mg/ml DNA) and ensure maximal recovery of the aqueous phase during each round of extraction.

For specific questions or concerns, please contact Mirus Bio Technical Support at 888.530.0801 or techsupport@mirusbio.com
For a list of citations using Mirus Bio products, please visit Technical Resources at www.mirusbio.com.

4.0 RELATED PRODUCTS

In Vivo* Gene Delivery Kits:

*Trans*IT®-*In Vivo* Gene Delivery System (Product # MIR 5100)

For DNA Tracking Studies:

Label IT® Tracker™ Intracellular Nucleic Acid Localization Kit (Product # MIR 7010,7011,7012,7013,7014,7015)

For Determination of Gene Expression Efficiency:

Beta-Gal Staining Kit (Product # MIR 2600)

Transfection Reagents:*

*Trans*IT®-LT1 Transfection Reagent (Product # MIR 2300)
*Trans*IT®-LT2 Transfection Reagent (Product # MIR 2400)
*Trans*IT®-Express Transfection Reagent (Product # MIR 2000)
*Trans*IT®-HeLaMONSTER® Transfection Kit (Product # MIR 2900)
*Trans*IT®-293 Transfection Reagent (Product # MIR 2700)
*Trans*IT®-Keratinocyte Transfection Reagent (Product # MIR 2800)
*Trans*IT®-CHO Transfection Kit (Product # MIR 2170)
*Trans*IT®-3T3 Transfection Kit (Product # MIR 2180)
*Trans*IT®-COS Transfection Kit (Product # MIR 2190)
*Trans*IT®-Insecta Transfection Reagent (Product # MIR 2200)
*Trans*IT®-Jurkat Transfection Reagent (Product # MIR 2120)
*Trans*IT®-Prostate Transfection Kit (Product # MIR 2130)
*Trans*IT-Neural® Transfection Reagent (Product # MIR 2140)
*Trans*IT-TKO® siRNA Transfection Reagent (Product # MIR 2150)
*Trans*IT®-siQUEST™ siRNA Transfection Reagent (Product # MIR 2110)
*Trans*IT®-Oligo Transfection Reagent (Product # MIR 2160)

RNA Interference Products:*

*Trans*IT-TKO® siRNA Transfection Reagent (Product # MIR 2150)
*Trans*IT®-siQUEST™ siRNA Transfection Reagent (Product # MIR 2110)
siXpress® PCR Vector Systems (Product # MIR 7300, 7301, 7302)
*Trans*IT-TKO® HTS-96 Plates (Product # MIR 2530, 2540, 2550, 2560, 2570)
Label IT®siRNA Tracker Intracellular Localization Kit with *Trans*IT-TKO® Transfection Reagent (Product # MIR 7200,7201,7202,7203,7204,7205)
Label IT®siRNA Tracker Intracellular Localization Kit with *Trans*IT®- siQUEST™ Transfection Reagent (Product # MIR 7206,7207,7208,7209,7210,7211)
Label IT®siRNA Tracker Intracellular Localization Kit (Product # MIR 7212,7213,7214,7215,7216,7217)

*These products are available in additional sizes.

The performance of this product is guaranteed for 6 months from the date of purchase if stored and handled properly.

Mirus Transfection Reagents are covered by United States Patent No. 5,744,335; 5,965,434; 6,180,784; 6,383,811, 6,593,465 and patents pending.

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