

Endotoxin Removal Kit User Protocol

Plasmid DNA Polishing Procedure:

1. If the starting DNA solution contains NaCl > 0.75M, dilute to < 0.5M with **DI Water**.
2. Dilute the starting DNA solution with an equal volume of **Mixing Buffer** in a 15 ml tube.
3. Fill a syringe with **Buffer C1 (POL)**, connect to a Perfluorosorb[®] S column and flush with all 5 ml of buffer.
4. Repeat Step 3 above.
5. Fill a syringe with DNA solution (from Step 2 above) and pass it through the column. Collect the flow-through, and repeat (two passes through the column). Push through at a rate of 1.5-2.5 minutes per column volume.
6. Fill the syringe with **Buffer C1 (POL)**, and flush the column with all 5 ml of buffer. Discard this syringe.
7. Fill a **NEW** syringe with **Buffer C2 (POL)** and wash the column with all 5 ml of buffer at a rate of 1.5-2.5 minutes per column volume.
8. Repeat Step 7 two more times (total wash is with 15 ml of Buffer C2).
9. Fill the syringe with **Buffer C3 (POL)**, and elute DNA from the column at a rate of 1.5-2.5 minutes per column volume. Collect the eluate in 2.5 ml fractions in 15 ml tubes.
10. Repeat step 9. Upon completion there will be 4 elution fractions. Measure the O.D. of each fraction at 260 nm. Most of the DNA will be in fractions 2 and 3, which should then be combined.

Please Note: All further manipulation of the DNA should be carried out with endotoxin-free reagents to reduce the risk of re-contamination.

Issue: 1a

Plasmid DNA Endotoxin Removal Kit

The ProMetic BioSciences Plasmid DNA Endotoxin Removal Kit incorporates Perfluorosorb[®] S, a specifically engineered adsorbent for purification of plasmid DNA by reverse phase chromatography. Perfluorosorb[®] S has a highly porous structure with high capacity for plasmid DNA and has no affinity for endotoxin.

This easy-to-use, highly efficient DNA polishing kit is designed to remove endotoxin from a DNA preparation with a starting endotoxin level of more than 3,000 EU/mg DNA and reduce the endotoxin to less than 1.0 EU/mg DNA, with a capacity for pDNA of approximately 0.5mg per prep.

Kit Contents:

- 10 x 5 ml syringes
- 5 x Perfluorosorb[®] S columns
- Mixing Buffer 25 ml
- Buffer C1 (POL) 100 ml
- Buffer C2 (POL) 100 ml
- Buffer C3 (POL) 100 ml

Additional Materials Needed:

- 50 ml tube
- 15 ml tubes
- High quality deionized water (DI Water)
- Spectrophotometer

Please see other side for User Protocol

International Sales and Technical Support:

ProMetic BioSciences (USA) Inc.
155 Willowbrook Boulevard, Suite 460
Wayne, NJ 07470, USA
Tel: +1.973.812.9880
Fax: +1.973.812.9881
E-mail: sales@prometic.com
techsupport@prometic.com