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ProductInformation

Endotoxin Removal Solution

Catalog Number **E4274**

Product Description

Endotoxins are lipopolysaccharides (LPS), a major component of the Gram-negative bacterial cell wall, and are commonly found as contaminants in plasmid DNA preparations from *E. coli.* Endotoxins are large, negatively charged molecules that co-purify with DNA on ion exchange and size exclusion columns and in CsCl banding. Endotoxins are extremely potent stimulators of the mammalian immune system and are toxic to primary cells and to animals. The endotoxin toxicity is an obstacle to *in vitro* and *in vivo* transfection experiments.

Non-ionic detergents, traditionally used for separation of integral membrane proteins,[1] can be utilized for removal of endotoxins from DNA solutions by phase separation.[2]

The solubility behavior of a detergent in a dilute, aqueous solution at physiological salt and pH conditions is strongly dependent upon the temperature of the solution. At low temperatures, the detergent forms a clear, micellar solution, but above the cloud point temperature, the micelles form larger, turbid aggregates and ultimately fuse to form a separate phase. The lower phase is detergent-enriched and the detergent-depleted upper phase contains detergent at a concentration slightly above the critical micellar concentration (CMC). Amphiphilic and hydrophobic molecules associated with the micelles of the detergent will aggregate within the detergent-enriched phase, while the soluble, hydrophilic molecules will remain in the detergent-depleted upper phase.

Extraction of endotoxin contaminated DNA solutions with the appropriate non-ionic detergent will separate the hydrophilic DNA from the amphiphilic endotoxin. The amphiphilic endotoxin will associate with the lower phase, while the DNA will remain in the upper, detergent-depleted phase.[2]

Reagents and equipment required, but not provided

- Water, Molecular Biology Reagent, Catalog Number W4502
- E-TOXATE® Water, Catalog Number 2107, or Tris-EDTA (TE) buffer 100×, Catalog Number T9285
- DNA solution (0.5 ml), ~ 1 mg/ml in E-TOXATE® Water or TE buffer
- 3 M sodium acetate solution, pH 7.5.
- 2-Propanol, Catalog Number I9516, or Ethanol, 190 proof, Catalog Number E7148; 200 proof, Catalog Number E7023
- 70% Ethanol
- E-TOXATE® reagents Kits, Catalog Numbers 210A1, 210B1 or 210C1
- Ice bucket
- Heat block or incubator at 37 °C
- Microcentrifuge at room temperature
- 1.5 or 2 ml sterile microcentrifuge tubes
- Endotoxin-free pipet tips (40-200 µl, 200-1000 µl)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage

Store at room temperature.

Note: Removal of endotoxins from DNA preparations can be performed either during the final stage of DNA preparation, or during an earlier stage.

Procedures for Endotoxin Removal

During the final stage of DNA preparation

Note: The procedure described below was performed on plasmid DNA produced in *E. coli* DH5α cells.

- Losses of up to 50% of the DNA are expected.
- Use of a DNA concentration above the recommended 1 mg/ml reduces the efficiency of the procedure.

1. Pipette 500 µl of the DNA solution into a sterile microcentrifuge tube.
2. Add 50 µl of the 3 M sodium acetate solution to the DNA sample.
3. Incubate on ice for 5 minutes.
4. Add 100 µl of cold Endotoxin Removal Solution.
5. Mix thoroughly and incubate on ice for 10 minutes. The solution should be light blue and clear.
6. Incubate the tube at 37 °C for 20 to 30 minutes or until the phases separate.
7. Spin for 5 minutes at 3000 x *g* in the microcentrifuge. The upper phase is colorless and clear, while the lower phase is blue.
8. Carefully transfer the upper phase containing the DNA to a clean microcentrifuge tube.
9. Repeat steps 4 through 8 twice.
10. Add 0.6× volume of 2-propanol. Mix by inversion at room temperature and centrifuge at 15,000 x *g* for 30 minutes at 4 °C. Alternatively, add 2.5× volumes of ethanol. Incubate overnight at –20 °C or 20 minutes at –70 °C and centrifuge at 15,000 x *g* for 30 minutes at 4 °C.
11. Carefully remove the supernatant
12. Wash the DNA pellet twice with cold 70% ethanol. Remove the supernatant.
13. Air-dry the pellet.
14. Suspend the DNA in 100 µl of endotoxin free water or TE buffer.
15. Determine DNA concentration and endotoxin levels using endotoxin assay reagents and compare to the starting material.

During an earlier stage of DNA preparation

This procedure is based on the alkaline lysis of *E. coli* DH5α cells.[3] The endotoxins are removed immediately after alkaline cell lysis, neutralization, and a clarification step. The resulting high salt solution is suitable for the endotoxin removal step. It is performed under "endotoxin free" conditions. The plasticware used is either sterile and disposable, or NaOH-treated. The buffers are prepared with endotoxin free water.

1. Add the Endotoxin Removal Solution (0.2× volume) to the cold, crude DNA solution.
2. Incubate on ice and mix occasionally by inversion to obtain a homogenous, clear blue solution
3. Incubate at 37 °C for 20 to 30 minutes until the phase separation is obvious.
4. Spin for 5 minutes at low speed (3000 x *g*) at room temperature.
5. Transfer the upper aqueous phase to an endotoxin free container.
6. Proceed with the DNA purification by any method. Use endotoxin-free buffers and containers.

References

1. Bordier, C., *J. Biol. Chem*., **256**, 1604-1607, (1981).
2. Cotten, M. *et al*., *Gene Therapy*, **1**, 239-246, (1994).
3. Sambrook *et al*., Molecular Cloning, a Laboratory Manual, 2nd Ed. p. 1.38

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