BcMag™ Quick Endotoxin Removal Kit

Introduction

Endotoxins, also known as Lipopolysaccharides (LPS), are a type of pyrogen with large complex molecules. They consist of an innermost core of hydrophobic fatty acid groups and a central and outermost region composed of hydrophilic polysaccharides (Fig.1). They are part of the outer membrane of the cell wall of gram-negative bacteria pathogens (such as Escherichia coli, Salmonella, Shigella, Pseudomonas, Neisseria, Haemophilus influenzae, Bordetella pertussis, and Vibrio cholera). They release into the circulation upon disrupting the intact bacteria (death, cell lysis).

The endotoxins are major contaminants found in commercially available biological products, which often adversely affect the study of the biological effects of the main ingredient. Gram-negative bacteria (*e.g., Escherichia coli*) are widely used in the biotechnology industry to produce recombinant products such as proteins, plasmid DNAs, and vaccines. These products can be contaminated with endotoxins at any point within the process. Removing Endotoxin from the product is critical since it can result in multiple pathophysiological effects, such as fever, shaking chills, septic shock, toxic pneumonitis, and respiratory symptoms lethality.

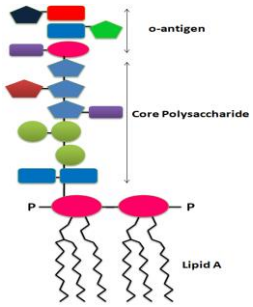

Fig. 1 Endotoxin structure

Removing endotoxin is one of the most challenging downstream processes during protein or DNA purification. Several methods are used to reduce endotoxin contamination of biological sample preparations, including affinity chromatography, such as immobilized polymyxin B, L-histidine, and poly-L-lysine, anion-exchange chromatography, gel filtration, ultrafiltration, sucrose gradient centrifugation, and Triton X-114 phase separation. The success of these techniques in reducing endotoxin contamination from a biological sample is strongly dependent on the properties of the target molecules. For example, ultrafiltration and ion exchange chromatography are commonly used techniques for removing endotoxin contaminants. Although ultrafiltration effectively removes endotoxins from water, it is not suitable for protein solution since the physical forces can damage the protein. Anion exchangers can effectively remove the Endotoxin but cause a significant loss of biological material due to adsorption. Many commercially available products are made from traditional chromatography matrices such as agarose resin or column. These solid matrices make the endotoxin removal process tedious, time-consuming, unable to handle very tiny samples, and challenging to adapt to the automation system. Bioclone introduces a powerful magnetic beads-based endotoxin removal system to overcome these problems.

BcMag™ Quick Endotoxin Removal Kit uses magnetic microsphere covalently immobilized with a high density of polymyxin B to remove endotoxin. It is specially designed for quick Endotoxin removal from various sample types. Polymyxin B, a peptide antibiotic, has a very high binding affinity for the lipid A moiety of most endotoxins. The microspheres combine all the advantages of affinity protein purification (low costs, simplicity, high specificity, and capacity) and magnetic properties to perform efficient manual or automatic quick high-throughput Endotoxin removal.

Workflow (Fig.2)

The purification with magnetic microparticles is straightforward.

1. Mix the microparticles with the sample and incubate them with continuous rotation for a sufficient time. During mixing, the beads remain suspended in the sample solution, allowing the endotoxins to bind to the immobilized polymyxin B.
2. After incubation, the beads are collected and separated from the sample using a magnet magnetic rack. Transfer the endotoxin-free supernatant to a fresh tube.

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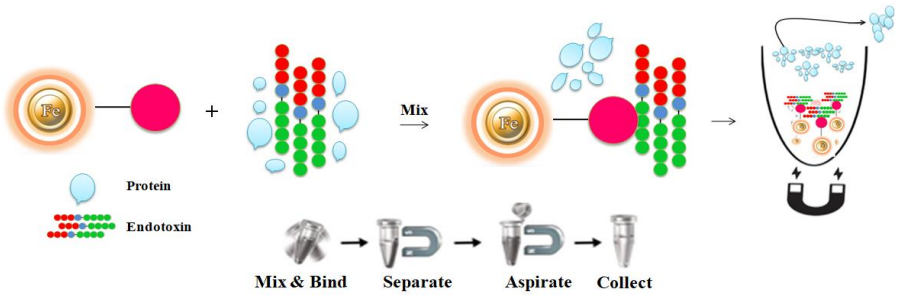

Fig.2 Endotoxin removal workflow

Specificities	
Composition	Magnetic microsphere immobilized with Polymyxin B
Magnetization	~60 EMU/g
Type of Magnetization	Superparamagnetic
Effective Density	2.5 g/ml
Concentration	50 mg/ml (dH_2O)
Binding Capacity	≥ 9,995 EU (endotoxin units) / ml
Storage	Ship at room temperature, Store at 4°C upon receipt

Protocol

Materials Required

- Regeneration Buffer: 1% Sodium deoxycholate
- Endotoxin-free dH_2O
- Magnetic rack (for manual operation)

  Based on sample volume, the user can choose one of the following magnetic racks: BcMag™ magnetic rack-2 for holding two individual 1.5 ml centrifuge tubes (Cat. # MS-01); magnetic rack-6 for holding six individual 1.5 ml centrifuge tubes (Cat. # MS-02); BcMag™ magnetic rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Cat. # MS-03); BcMag™ magnetic rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Cat. # MS-04); BcMag™ magnetic rack-96 for holding a 96 ELISA plate or PCR plate (Cat. # MS-05). For larger scale purification, Ceramic magnets Block for large scale purification (6 in x 4 in x 1 in block ferrite magnet, Applied Magnets, Cat# CERAMIC-B8)

A. Procedure

Note:

- *Equilibrate all reagents and samples to room temperature because temperature, pH, and ionic strength affect the performance of the Particles.*
- *To minimize nonspecific binding, adjust all buffers to pH 7-8 and salt concentration 0.1-0.5 M NaCl (final concentration), although the Particles can bind to LPS at pH 5-9.*
- *Use only endotoxin-free solutions to prevent introducing any endotoxin into the sample.*

1. Vigorously shake the bottle until the magnetic beads become homogeneous and transfer an appropriate volume of the magnetic to a new tube or flask.

   Note:

   - *Optimize the number of beads used for each application. Do not allow the beads to sit for more than 3 minutes before dispensing. Resuspend the magnetic*

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2. Place the tube on the magnetic rack for 1-3 minutes until the supernatant becomes clear. Remove the supernatant while the tube remains on the magnetic rack.
3. Add ten particle-bed volumes of endotoxin-free H_2O and mix the particles by pipetting or vortex. Again, place the tube on the magnetic rack for 1-3 minutes and remove the supernatant while the tube remains on the magnetic rack.
4. Repeat step (3) one more time.
5. Resuspend the particles with ten particle-bed volumes of regeneration buffer and incubate at room temperature for 15 minutes with continuous rotation.
6. Wash the particles with F particle volumes of a suitable endotoxin-free buffer or dH_2O three times as described in step (2).
7. Add an appropriate amount of protein or DNA solution to the particles and incubate at room temperature for 15 minutes with continuous rotation.
8. Place the tube on the magnetic rack for 1-3 minutes until the supernatant becomes clear. Remove the supernatant to an endotoxin-free tube while the tube remains on the magnetic rack.

B. Particles reuse

Note:

- *For the same protein or DNA, it is possible to reuse the particles.*
- *Regenerated particles may be used at least 5 times without loss of activity.*
- *The Particles must be regenerated before each use, including first-time use.*

1. Regenerate particles by washing the particles with five particle-bed volumes of regeneration buffer three times to remove any bound endotoxin as described in step A (2).
2. Wash the particles with five particle-bed volumes of endotoxin-free dH_2O three times as described in step A (2).
3. Store the particles in 20-25% ethanol at 2-8°C.

Related Products	
Product Name	Product Name
One-Step Lipids Removal Kit	Quick Albumin Removal Kit
One-Step Deproteinizing Kit	Quick HSA and IgG Depletion Kit
One-Step SDS Removal Kit	One-Step Dye Removal Kit
One-Step Detergent Removal Kit	Quick Endotoxin Removal Kit
EDTA Metal Ion removal Kit	Immobilized TCEP Disulfide Reducing Kit
EGTA Metal Ion removal Kit	One-Step PCR Inhibitor Removal Kit
One-Step DNA and RNA Cleanup Kit	One-Step DNA and RNA Removal Kit
One-Step Sequencing Cleanup Kit	One-Step Single-Stranded DNA Removal Kit
One-Step Fluorescent Labeling Cleanup Kit	One-Step RNA Removal Kit
One-Step NGS Cleanup Kit	One-Step PCR Cleanup Kit

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