CREATIVE BIOMART®

Endotoxin Removal Kit Protocol

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Product Name: Endotoxin Removal Kit, Magnetic beads
Cat. No.: Endotoxin Removal-002MB

I. Description

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. Creative BioMart introduces a powerful magnetic beads-based endotoxin removal system to overcome these problems.
It is a highly efficient endotoxin removal kit, which is fixed on polymer magnetic microspheres with endotoxin binding protein as the ligand. Due to its magnetic properties, it is easy to separate and remove after specific endotoxin removal. The final endotoxin of samples purified by this endotoxin removal kit is less than 0.25EU/ml.

Table 1. Endotoxin Removal Kit specifications:

Specificities	
Composition	Magnetic microsphere
Ligands	Endotoxin binding protein
Range of pH	4.5-8.5
Concentration	10 mg/ml
Binding Capacity	20000-25000EU (endotoxin units) / ml
Storage	Ship at room temperature, Store at 4°C upon receipt
Storage Buffer	Store at 0.01M PBS(pH=7.4).

II. Key Features

1. This kit can remove the low endotoxin residues in the sample. In samples containing 4000EU/ml, 400EU/ml, 40EU/ml and 4EU/ml, the endotoxin can be removed to less than 0.25EU/ml at one time with this kit.
2. The removal steps are simple, just wash the endotoxin removal magnetic beads, add them directly to the sample, mix and incubate for 30 minutes, and then use the magnetic rack to remove the endotoxin in the sample.
3. Does not affect the properties of the sample, this product specifically adsorbs endotoxin, and the adsorption conditions are mild, ensuring the product recovery rate ≥ 95%.
4. Easy to use, the kit provides a non-pyrogenic washing buffer, and non-pyrogenic pipette tips.

III. Kit Contents

Kit contents (Cat. No.: Endotoxin Removal-002MB)	PK
Magnetic beads	1mL
Washing Buffer	5mL
Non-pyrogenic tips-200ul	10
Non-pyrogenic tips-1000ul	10
Protocol	1

IV. Materials and Equipment required but not Provided

1. Magnetic rack (for manual operation)

V. Endotoxin Removal Protocol

Cleaning and equilibration of magnetic microspheres

1. Calculate the volume of endotoxin removal magnetic beads that need to be added according to the endotoxin content. 1ml endotoxin removal magnetic beads can remove 20000-25000EU endotoxin, and the concentration of endotoxin removal magnetic beads is 10mg/ml;
2. Vigorously shake the bottle until the magnetic beads become homogeneous, do not allow the beads to sit for more than 3 minutes before dispensing. Use a non-pyrogenic pipette tip to pipette the corresponding volume of endotoxin-removing magnetic beads. 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 rack. Add 5 particle-bed volumes of Wash Buffer to resuspend the magnetic beads, 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 rack;

Endotoxin removal

Wash the particles with Wash Buffer 3-5 times. Add an appropriate amount of protein or DNA solution to the particles and incubate at 4°C or room temperature for 30-60 minutes with continuous rotation. 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 rack.

Notes:

1. Endotoxin removal magnetic beads have a strong binding ability to endotoxin, and it is recommended to use it in a sterile environment, such as a biological safety cabinet or an ultra-clean bench;
2. Use a non-pyrogenic pipette tip to draw any reagent in the kit to prevent introducing any endotoxin into the sample;
3. 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 4.5-8.5;
4. If the sample is relatively stable, such as a plasmid sample, it can be incubated at room temperature, but if the sample is not stable, it is recommended to remove endotoxin at 4°C, and the mixing process should be as gentle as possible. The shaker shakes slowly from side to side. It is not recommended to use a rotator to shake up and down, so that the protein is easy to precipitate.;
5. 1ml of endotoxin filler can remove 20000-25000EU of endotoxin under the premise that the sample and endotoxin removal filler are fully incubated. Therefore, if a large volume of sample is used, it is recommended that the volume of endotoxin removal magnetic beads be appropriately increased to ensure sufficient mixing.

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