

Bioneer Corporation  
8-11 Munpyeongseo-ro, Daedeok-gu, Daejeon, 34302  
Republic of Korea  
Tel: +82-42-930-8777 (Korea : 1588-9788)  
Fax: +82-42-930-8688 E-mail: sales@bioneer.com

Bioneer Inc.  
155 Filbert St. Suite 216  
Oakland, CA 94607, USA  
Toll Free: +1-877-264-4300 Fax: +1-510-865-0350  
E-mail: order.usa@bioneer.us.com

Bioneer R&D Center  
Korea Bio Park BLDG #B-702, 700 Daewangpangyo-ro  
Bundang-gu, Seongnam-si, Gyeonggi-do, 13488  
Republic of Korea  
Tel: +82-31-628-0500 Fax: +82-31-628-0555

## 1. Description

Endotoxin (LPS) is one of constituent of cell wall of Gram-negative causes cell toxicity and immune reaction. ToxinCleanic Endotoxin Removal Resin consists of ToxinCleanic Endotoxin removal resin and 10X Regeneration buffer. The resin made from agarose is conjugated with polymyxin B sulfate that is a family of antibiotics. The resin is offered 50% slurry in 20% ethanol solution. The resin can remove the LPS in protein sample up to 95%. The resin can be packed into columns and remove the LPS from sample by gravity or centrifuge.

## 2. Components

Kit components	Cat. No. K-7800
Endotoxin removal resin	10 ml
10X Regeneration buffer	10 ml
Manual	1 ea

\*10X Regeneration buffer is 10 % Sodium deoxycholate in Endotoxin free water.

## 3. Materials to be supplied by user

Components
Endotoxin free water
Endotoxin free plastic wares
Disposable column
Centrifuge

## 4. Product characteristic

Product Specificities	
Composition	Agarose resin
Concentration	50% slurry
Binding capacity	> Decrease 90%
Storage buffer	20% EtOH
Storage condition	2 - 8°C

## 5. Experimental Procedure

- ☞ Amount of resin and buffer can be altered depending on user's needs or convenience.
- ☞ Dilute 10X Regeneration buffer to 1X.
- ☞ Regenerate the resin before each use.
- ☞ Recommend degassing the resin and buffer before applying the column to prevent air bubbles from clogging the column.
- ☞ Use the Endotoxin free plastic wares and tips.

### 5.1 Endotoxin removing - gravity flow

#### Preparation

1. Resuspend Endotoxin removal resin by gentle vortexing.
2. Transfer 1 ml of Endotoxin removal resin into 2 ml column and allow the resin to settle.
3. Wash the resin by adding 2 ml of Endotoxin free water to remove the storage buffer.
4. Repeat the step 3, 2 more times.
5. Add 2 ml of 1X Regeneration buffer to the resin for regeneration.
6. Wash the resin by adding 2 ml of Endotoxin free water to remove the 1X Regeneration buffer.
7. Repeat the step 6, 2 more times.

#### Endotoxin removal

1. Transfer the sample to the column containing the resin.
2. Collect the flow through in Endotoxin free tube.
3. Endotoxin level can be checked by using Endotoxin assay kit.
4. If necessary, repeat the endotoxin removal procedure by reloading the sample to column.

### 5.2 Endotoxin removing - spin column

#### Preparation

1. Resuspend Endotoxin removal resin by gentle vortexing.
2. Transfer 500 µl of Endotoxin removal resin into 1.5 ml tube.
3. Centrifuge the tube at 500 x g for 1 min to remove the storage buffer.
4. Transfer 1 ml of Endotoxin free water to the tube to wash the resin.
5. Centrifuge the tube 500 x g for 1 min and remove the Endotoxin free water.
6. Repeat the step 4 - 5, 2 more times.
7. Add 1 ml of 1X Regeneration buffer to the resin for regeneration.
8. Centrifuge the tube 500 x g for 1 min and remove the Regeneration buffer.
9. Transfer 1 ml of Endotoxin free water to the tube to wash the resin.
10. Centrifuge the tube 500 x g for 1 min and remove the Endotoxin free water.
11. Repeat the step 9 - 10, 2 more times.

#### Endotoxin removal

1. Transfer the sample to the tube containing the resin.

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2. Collect the supernatant in Endotoxin free tube.
3. Endotoxin level can be checked by using Endotoxin assay Kit.
4. If necessary, repeat the endotoxin removal procedure by reloading the sample to column.

## 6. Troubleshooting

### 6-1. Removal efficiency is low

- ☞ Adjust the pH of sample to neutral range.
- ☞ The sample is contaminated by extrinsic endotoxin.
- ☞ Resin has to regenerate before each use.

### 6-2. Sample loss is high

- ☞ Add Sodium chloride (NaCl) to decrease the non-specific binding.

## 7. Notice

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