ToxinEraser™ Endotoxin Removal Resin **Cat. No. L00402**

Technical Manual No. 0366 **Version 10112010**

I. DESCRIPTION

Lipopolysaccharide (LPS) is a bacterial endotoxin, and a major constituent of the cell walls of gram-negative bacteria. During gram-negative bacteremia or endotoxemia, LPS is the principal pathophysiological mediator by which bacteria can cause hypotension, organ failure, disseminated intravascular coagulation, and fatal shock in mammalian hosts. The removal of these endotoxins is highly necessary for downstream processes.

The ToxinEraser™ Endotoxin Removal Resin utilizes immobilized polymixin B to bind and remove pyrogens from solution. The polymixins are a family of antibiotics that contain a cationic cyclopeptide with a fatty acid chain. Polymixin B neutralizes the biological activity of endotoxins by binding to the lipid A portion of bacterial lipopolysaccharide.

The characteristics of the resin are listed in table 1.

Table 1. Characteristics of ToxinEraser™ Endotoxin Removal Resin

Binding Capacity	Up to 2,000,000 EU/ml resin.
Ligand	Modified PMB (Polymixin B)
pH Stability	pH 5 -10
Support Matrix	4% cross-linked agarose, spherical beads
Mean Particle Size	90 μm
Storage	2°C to 8°C (Do not freeze.)
Shelf Life	12 months
Types of substances that can be applied to the column	Protein, including peptides and antibody, polysaccharide, etc.
Applicable Ionic Strength	0.1 to 0.5 M NaCl
Substances tested that do not interfere with performance	20% DMSO, 20% ethanol, 20% glycerol; 1 M urea, 300 mM imidazole; 0.05% Tween 20, 10 mM DTT, etc.

II. KEY FEATURES

- High stability and high removal efficiency.
- High binding capacity: > 2, 000, 000 EU / ml (CV).
- Reusable up to five times if properly regenerated.

III. SIZE

The beads are supplied in 2 ml of 50% slurry with a drained volume or column volume (CV) of 1 ml.

IV. MATERIALS AND EQUIPMENT

1. Column, column stand.
2. 0.1 N sodium hydroxide or 0.1 N hydrochloric acid for adjusting the pH of the sample.
3. Regeneration Buffer. For regeneration of the resin, Genscript Regeneration Buffer is needed. The Cat. No. of this product is M01053.
4. Equilibration Buffer, customer can use any proper buffer to equilibrate the resin. A pH of 7-8 and the salt concentration of 0.15-0.5 M NaCl are recommended for best results.

V. Endotoxin Removal Protocol

Notes:

- ToxinEraser™ Endotoxin Removal Resin must be regenerated before each use, including first use.
- Use only pyrogen-free solutions and materials to prevent introducing additional endotoxin into the sample.
- Degas all solutions before applying to the column to prevent air bubbles from clogging the column and reducing flow.
- Equilibrate all solutions and resin to room temperature before use.

Sample Preparation. pH and ionic strength of the samples are the main factors that affect the performance of the resin. In general, a pH of 7-8 is recommended, although the binding of LPS to the resin occurs from pH 6 to 9. Proper ionic strength is necessary to decrease the nonspecific binding or the loss of protein. 0.15-0.5 M NaCl is recommended for high–efficiency endotoxin removal and lower sample loss. Adjust sample pH and ionic strength with NaCl, 0.1N sodium hydroxide or 0.1N hydrochloric acid.

1. Column Activation. Mix the slurry by gently inverting the bottle several times to resuspend the resin completely. Use a pipette to transfer 1 ml of ToxinEraser™ Endotoxin Removal Resin slurry to a column. Allow the resin to settle and the storage buffer to drain from the column. Wash the column by adding 3 ml of Regeneration Buffer (GenScript, Cat. No. M01053) and let the buffer drain completely. Set the flow rate at 0.25 ml/min or less than 10 drops per minute by adjusting the flow-speed control. Repeat the wash step two more times to make this system endotoxin-free. It is important to rinse the wall of the column from top to bottom using regeneration buffer.

2. Column Equilibration Equilibrate the column by adding 3 ml of equilibration buffer (GenScript, Cat. No. M01054) and let the buffer drain completely at a speed of 0.5 ml/min. Also, the column wall should be rinsed completely during this process. Repeat the equilibration step two more times.

3. Endotoxin Removal. Apply the sample to the column. Set the flow rate at 0.25 ml/min or 10 drops per minute by adjusting the flow-speed control. Start collecting the sample after a void volume of 1 ml. After sample completely gets in the column, add 1 ml of equilibration buffer or the same buffer for sample to recover the sample. Pool the fractions containing protein sample and detect the endotoxin in it.

4. Reloading of the Sample. If the final endotoxin level is above the desired endotoxin level. Repeat the endotoxin removal procedure by reloading the sample to the regenerated column.

VI. TROUBLESHOOT

Problem	Possible Cause	Solution
The removal efficiency is low.	The pH of the sample is not within pH 7-8 range.	Adjust to pH 7-8.
	The contact time between sample and the resin is too short.	Increase the contact time by decreasing the flow rate.
	The removal or detection system is contaminated by extrinsic LPS.	Use endotoxin-free labware.
	LPS binds to target protein strongly.	1. Optimize the pH of sample buffer for disaggregation. 2. Increase the contact time by decreasing the flow rate. .
The loss of sample is high.	There is non-specific binding of sample to resin.	Increase the concentration of NaCl in the sample and equilibration buffer.
	The target protein is aggregated with LPS and is removed with it.	1. Optimize the pH of sample buffer for disaggregation. 2. Increase the contact time by decreasing the flow rate. .
The sample is contaminated.	The column has already been used for another sample.	Do not use the column for samples containing different target molecules.

VII. ORDERING INFORMATION

ToxinEraser™ Endotoxin Removal Resin (2 ml of 50% slurry):	Cat. No. L00402
ToxinEraser™ Regeneration Buffer:	Cat. No. M01053
ToxinEraser™ Equilibration Buffer:	Cat. No. M01054

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