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ToxinEraser™ Endotoxin Removal Resin

Cat. No.: L00402

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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 polymyxin B to bind and remove pyrogens from solution. The polymyxins are a family of antibiotics that contain a cationic cyclopeptide with a fatty acid chain. Polymyxin 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 (Polymyxin 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)
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

1. High stability and high removal efficiency.
2. High binding capacity: > 2, 000, 000 EU / ml settled resin.
3. Reusable up to five times if properly regenerated.

III. Materials and Equipment Not Provided

1. Column, column stand.
2. 0.1 M sodium hydroxide or 0.1 M hydrochloric acid for adjusting the pH of the sample.
3. 3M NaCl for adjusting the ionic strength of the sample.
4. Regeneration Buffer. For regeneration, GenScript Regeneration Buffer is needed. The Cat. No. of this product is M01053.
5. Equilibration Buffer. For equilibration, GenScript Regeneration Buffer is needed. The Cat. No. of this product is M01054. Customer can use any proper buffer to equilibrate the resin. A buffer with pH of 7-8 and 0.15-0.5 M NaCl are recommended for best results.
6. Pyrogen-free collection tube and tips.

IV. Endotoxin Removal Protocol

Notes:

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

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, 0.1M sodium hydroxide or 0.1M hydrochloric acid.

Endotoxin removal

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 (steps 1-3) by reloading the sample to the regenerated column.

V. Troubleshooting

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.	<ol style="list-style-type: none"> 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.	<ol style="list-style-type: none"> 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.

VI. Ordering information

Cat.No.	Product name
L00338	ToxinEraser™ Endotoxin Removal Kit
M01053	ToxinEraser™ Regeneration Buffer
M01054	ToxinEraser™ Equilibration Buffer
M01063	ToxinSensor™ Endotoxin-free Pipette Tips (1 ml, Blue)

For research and manufacturing use. Direct human use, including taking orally and injection are forbidden.

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