**ToxinEraser™ Endotoxin Removal Kit** **Cat. No. L00338**

**Technical Manual No. 0312** **Update date 08032009**



## 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 but also highly difficult.

GenScript ToxinEraser is an endototoxin removal resin of high efficiency. It is based on the affinity matrix of modified polymyxin B (PMB), which allows highly efficient endotoxin removal. The final endotoxin level can be decreased to less than 0.1 EU/ml with repeat use of ToxinEraser™ endotoxin removal resin. The characteristics of the resin are listed in table 1.

**Table 1. Characteristics of ToxinEraser™ Endotoxin Removal Resin**

| | |
|---|---|
| Size | 1.5 ml in pre-packed column |
| 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 | 18 months |
| Equilibration Buffer | Phosphate-Buffer, pH 8.0 |
| 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;<br>1 M urea, 300 mM imidazole;<br>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)
- Fast flow without any constant speed pump
- Reusable up to five times if properly regenerated
- Ready-to-use reagents and materials, such as equilibration buffer, collection tubes, etc.

## III. CONTENTS

| **Kit Contents (Cat. No. L00338)** | **3 - 5 Assays** |
|---|---|
| ToxinEraser™ Endotoxin Removal Resin | 1.5 ml pre-packed column |
| Regeneration Buffer | 125 ml |
| Equilibration Buffer | 125 ml |
| Flow-Speed Control | 1 |
| Collection Tubes | 1 pack (3 per pack) |
| Tips (1 ml) | 2 packs (6 per pack) |
| Protocol | 1 |

Equilibration buffer and regeneration buffer are separately available from GenScript.

| | | |
|---|---|---|
| Regeneration Buffer | Cat. No. M01053 | 125 ml |
| Equilibration Buffer | Cat. No. M01054 | 125 ml |

## IV. MATERIALS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Column stand.
2. 0.1 N sodium hydroxide or 0.1 N hydrochloric acid for adjusting the pH of the sample, and 3M NaCl for adjusting the ionic strength of the sample.

## V. ENDOTOXIN REMOVAL PROTOCOL

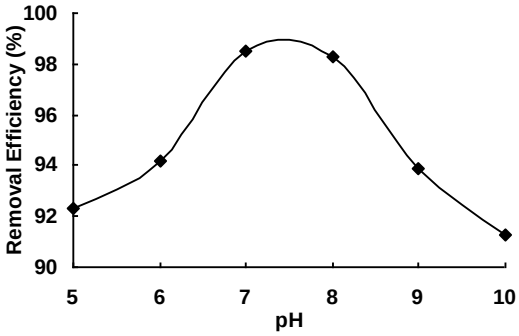
**Sample Preparation.** pH and ionic strength of the samples are the main factors that affect the performance of the resin. In general, a pH of 7- 8 is optimal, although the binding of LPS to the resin occurs from pH 6 to 9. Proper ionic strength can reduce the nonspecific binding or the loss of protein. 0.15 - 0.5 M NaCl is recommended for high–efficiency endotoxin removal and lower sample loss.

1. **Column Activation.** Place the column upright in the stand. Remove the top cap first to prevent bubbles from being drawn into the gel. Allow storage solution to drain completely from the column, but do not allow the column bed to dry. Wash the column by adding 5 ml of cold regeneration buffer (**Do not warm it up, otherwise it will become cloudy**) and let the buffer drain completely. Set the flow rate at 0.25 ml/min or 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. This process may take approximately 60 minutes for a 1.5 ml column.
2. **Column Equilibration** Equilibrate the column by adding 6 ml of equilibration buffer 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. This process may take approximately 40 minutes.
3. **Endotoxin Removal.** Close the flow-speed control after column equilibration. 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 1.5 ml. After sample completely gets in the column, add 1.5 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.
5. **Storage of the Column.** For storage of the column, wash the column with 10 ml of equilibration buffer and allow the column to drain completely. Add 1.5 ml of regeneration buffer

supplemented with 0.02% sodium azide. Store at 2°C to 8°C. Do not freeze.

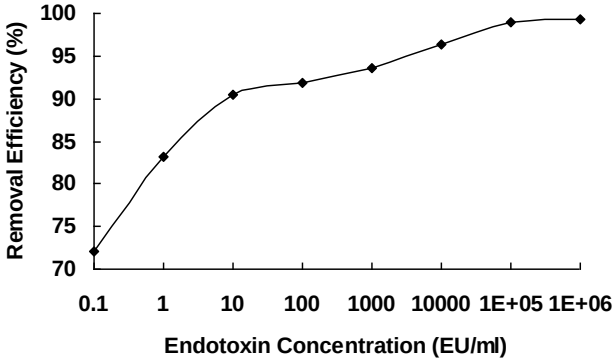
## VI. FACTORS AFFECTING ENDOTOXIN REMOVAL EFFICIENCY

Although the mechanism of the specific binding of PMB to LPS is still under discussion, it is universally believed that it is a cocktail effect of electrostatic interaction, ionic interaction, hydrophobic binding, and molecular recognition. Therefore, several parameters, such as the pH of the buffer, the concentration of LPS, the contact time, the ionic strength, the temperature, and the other properties of the sample of interest will affect the efficiency of endotoxin removal. Here are some examples:




**The Effect of pH on Removal Efficiency**

Fig. 1. Endotoxin removal can be performed in solution from pH 5 -10 but the highest removal efficiency occurs at pH 7-8. The removal efficiency decreases with the deviation from neutral pH.




**The Effect of Endotoxin Concentration on Removal Efficiency**

Fig. 2. Endotoxin removal efficiency is highly affected by and directly proportional to endotoxin concentration.

## VII. TROUBLESHOOTING

| Problem | Possible Cause | Solution |
|---|---|---|
| The removal efficiency is low. | The pH of the sample is not within pH 7 - 8 range. | Adjust pH 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.<br>2. Increase the contact time by decreasing the flow rate . |
| The sample loss 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 the sample to reduce aggregation.<br>2. Increase the contact time by reducing the flow rate . |
| The sample is contaminated. | The column has already been used for another sample. | Do not reuse the column for samples containing different target molecules. |
| The Regeneration Buffer is cloudy. | The Regeneration Buffer will become cloudy when warmed up to room temperature. | Cool the Regeneration Buffer on ice before use or perform the regeneration at 4°C. |

## VIII. ORDERING INFORMATION

ToxinEraser™ Endotoxin Removal Kit Cat. No. L00338

ToxinEraser™ Regeneration Buffer Cat. No. M01053

ToxinEraserTM Equilibration Buffer Cat. No. M01054

**For Research Use Only.**

**PATENT Pending.**

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