ToxinEraser™ Advanced Endotoxin Removal Kit Cat. No. L00408

Technical Manual No. 0453 **Update Date 07/08/2010**

I. DESCRIPTION

ToxinEraser™ Advanced Endotoxin Removal Kit is an endotoxin removal system based on an endotoxin binding protein (EBP) from the horseshoe crab (Limulus polyphemus). Endotoxin is the principal pathophysiological mediator of hypotension, organ failure, disseminated intravascular coagulation, and fatal shock in mammalian hosts. In tissue culture and animal experiments, high toxicity of endotoxins *in vivo* and *in vitro* can produce misleading and uninterruptable results, also valuable time consuming and money loss. Therefore, the removal of endotoxins from samples preparations is often necessary to the use of sample in downstream applications.

GenScript ToxinEraser™ Advanced Endotoxin Removal kit is designed for rapid endotoxin removal from proteins , peptides and antibodies, and even DNA samples. In addition, the kit has a higher endotoxin binding specificity for high-molecular hydrophobic proteins. The pH and NaCl concentration of the samples solution may be adjusted to the desired value for high recovery and removal efficiency. Once samples have had endotoxins removed with ToxinEraser™ Advanced Endotoxin Removal Kit, endotoxin levels are efficiently reduced to 0.1 EU/g or less.

Table 1. Characteristics of ToxinEraser™ EBP Endotoxin Removal Kit

Size	1.5 ml in pre-packed column
Binding Capacity	2,000,000 EU/ml resin
Ligand	Endotoxin Binding Protein (EBP)
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.)
Equilibration Buffer	Phosphate-Buffer, pH 8.0

II. KEY FEATURES

- High specificity and high removal efficiency (>90%)
- High binding capacity: 2, 000, 000 EU / ml (CV)
- High Recovery Yield: >90% with minimized sample loss
- Reusable up to five times without much activity loss if properly regenerated

III. CONTENTS

Kit Contents (Cat. No. L00408)	**3 - 5 Assays**
ToxinEraser™ Advanced Endotoxin Removal Resin with Flow-Speed Control	1.5 ml pre-packed column
Regeneration Buffer	125 ml
Equilibration Buffer (based on “Phosphate Buffer”, pH 7.4)	125 ml
Collection Tubes	1 pack (3 per pack)
Tips (1 ml)	2 packs (6 per pack)
Protocol	1

IV. ADDITIONAL MATERIALS REQUIRED

1. Column stand.
2. 0.1 N sodium hydroxide or 0.1 N hydrochloric acid for adjusting the pH of the sample.

V. ENDOTOXIN REMOVAL PROTOCOL FROM PROTEIN

1. Sample Preparation

Check the pH value of the sample solution and adjust to pH 7-8 with 0.1 M NaOH or 0.1 M HCl if necessary, although the binding of LPS to the resin occurs from pH 6 to 9. Adjust NaCl concentration in sample solution (recommended range 0.15M - 0.5M) with NaCl to decrease the non-specific binding.

Note: The pH and NaCl concentration of the samples may be adjusted to the desired value for high recovery and removal efficiency. However, any solution you use should be endotoxin-free.

2. Column Preparation

Column Activation.

Place the column upright in the stand, remove the top lid from column and it is necessary to prevent bubble formation in the column that would reduce the column's binding capacity and resolution, and allow storage solution to drain, but do not allow the column to run dry. After the column has drained, add 5 ml of regeneration buffer at a flow rate of 0.25 mL/min or 10 drops per minute by adjusting the flow-speed control, also, rinse the walls of the column with regeneration buffer, and it is necessary to repeat this step at least three times to make this system endotoxin-free. This process may take approximately 60 minutes for the column (1.5 ml) preparation.

Column Equilibration.

Adding 6 ml of equilibration buffer at a flow rate of 0.5 ml/min. It is important to rinse the wall of the column during this process, and repeat the equilibration process at least three times to make this system endotoxin-free. Total process may take approximately 40 minutes.

Note: Because Equilibration Buffer in the kit (L00408, GenScript) is based on Phosphate Buffer (pH 7.4). So if the sample is free of phosphate buffer, we recommend using sample buffer not equilibration buffer (prepared with endotoxin-free water) when equilibration process.

3. Endotoxin Removal

Loading Sample.

After column equilibration, close the flow-speed control, then add the sample (no more than 20 ml) to the column with pipette tips (LAL Reagent Grade). Set the flow rate at 0.25 ml/min or 10 drops per minute by adjusting the flow-speed control.

Collecting Sample.

Collect the flow-through (void volume) and elute the sample with 1.5 ml equilibration buffer or sample buffer. Pool the fractions (containing protein sample), then detect the endotoxin in the sample (We recommend using GenScript ToxinSensor™ Chromogenic LAL Kit for endotoxin detection, L00350).

Note: Because Equilibration Buffer in the kit (L00408, GenScript) is based on Phosphate Buffer (pH 7.4). So if the sample is free of phosphate buffer, we recommend using sample buffer not equilibration buffer (prepared with endotoxin-free water) when equilibration process.

4. Repeating Endotoxin Removal

Determine final endotoxin concentrations of samples. If the measured value is greater than desired endotoxin level, repeat the endotoxin removal procedure by reloading the activated column with the sample.

VI. QC Control

Product	Tested type of substance	Original Protein Concentration (mg/ml)	Original Endotoxin Level (EU/mg)	Final Endotoxin Level (EU/mg)	Protein Loss (%)	Endotoxin Removal Efficiency (%)
L00338	Protein	9.69	102	0.23	9.8	99.8
L00408				0.21	3.6	99.8
L00338	DNA	0.308	1450	8	20.1	99.4
L00408				7	7.1	99.5

VII. STORAGE

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 the column at 2°C to 8°C. Do not freeze the column.

VIII. TROUBLESHOOTING

Problem	Possible Cause	Solution
Low endotoxin removal efficiency .	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 to reduce the binding. 2. Increase the contact time by decreasing the flow rate.
High sample loss.	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. 2. Increase the contact time by decreasing the flow rate .
Sample contamination	The column has already been used for another sample.	Do not use the column for samples containing different target molecules.

IX. ORDERING INFORMATION

ToxinEraser™ Advanced Endotoxin Removal Kit Cat. No. L00408

For Research Use Only.

PATENT Pending.

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