Procedure for Endotoxin removal with EndoBind-R™

1. Remove the top cap.
2. Remove the bottom cap.
3. Allow the 0.02% sodium azide storage solution to drain from the column.
4. Wash the column with 50.0 ml endotoxin-free water to remove sodium azide.
5. Equilibrate the column with 5.0-10.0 ml of sample buffer.
6. Add 2.5-5.0 ml of sample to EndoBind-R™ and allow it to penetrate the column. Collect the flow-through in an endotoxin-free tube.
7. Add 5.0 ml of sample buffer to the column and collect flow-through. Repeat this step until a total of five 5.0 ml fractions have been collected.

Substances pass through the column at different rates, so it is important to check each fraction for endotoxin-free product. This can be done by measuring the absorbance of the flow-through fractions at OD_{280} for proteins and OD_{260} for DNA. The majority of protein and DNA generally pass through the column in the first two fractions. Check for endotoxin removal using an appropriate assay.
Refer to EndoBind-R™ Application Notes for guidance on proper sample buffer conditions.

Procedure for EndoBind-R™ Storage and Maintenance

After each use the EndoBind-R™ column should be washed before storage:

1. Rinse the column with 50.0 ml endotoxin-free water.
2. Wash the column with 10.0 ml of 2.0 M sodium chloride.
3. Rinse the column with 50.0 ml endotoxin-free water. This step is crucial to remove all traces of salt.
4. Wash the column with 10.0 ml of 0.5 N sodium hydroxide.
5. Rinse the column with 50.0 ml endotoxin-free water.
6. Add 20.0 ml of 0.02% sodium azide and store upright at 4°C.

In addition to routine cleaning, EndoBind-R™ shows optimal performance when each column is dedicated to the purification of a specific protein or DNA solution. The column is shipped in 0.02% sodium azide.

Product Characteristics	EndoBind-R
pH Range (buffer)	pH 5.0-9.0
Binding Capacity	2,000,000 EU/ml resin
Binding Affinity	10^{-7} - 10^{-8} M
Flow Rate	Gravity
Purity	>98% Factor C Sushi Peptide
Temperature Stability	Regular use between 4-30°C.

The Soluble Biotech suite of endotoxin removal technologies were developed in 2003 to create products for detection, removal and neutralization of bacterial toxins.

Endotoxin Removal Products:

EndoBind-R™	1 ml column	EBR-3001.01
EndoBind-R™	5 ml column	EBR-3005.01
EndoBind-R™	Bulk resin	Inquire

Soluble BIOTECH
A Predictive Oncology Company

200 Riverhills Business Park
Suite 250
Birmingham, AL 35242

E-mail: info@solublebiotech.com

www.solublebiotech.com

EndoBind-R™ Instruction Booklet

Advantages

- High binding affinity and capacity
- Non-cytotoxic; non-hemolytic
- Chemically-synthesized compound minimizes lot-to-lot variability
- No special buffers required for binding or washing
- Large pore size
- Hydrophilicity minimizes non-specific binding

Product Description

EndoBind-R™ is a 5.0 ml DADPA-agarose conjugated Sushi peptide affinity chromatography column bound to a 4% cross-linked beaded agarose support resin. It can be used to remove endotoxin from water, buffer and cell culture media. Under optimized conditions it can also remove endotoxin from protein and DNA samples. It can be used over a broad range of conditions with high specificity.

Introduction to Endotoxin

The removal of endotoxin from water, buffers, cell culture media, and protein and DNA preparations is a priority. The majority of lipid in the outer membrane of gram-negative bacteria consists of lipopolysaccharide (LPS), also called endotoxin. On average, a single *E. coli* cell contains 2,000,000 LPS molecules. Sub-nanogram levels of endotoxin can trigger immune responses and alter the phenotype and function of many cells including monocytes, neutrophils, dendritic cells, hepatocytes, vascular and respiratory epithelium, and arterial smooth muscle cells. Recently, a 34 amino acid Sushi domain was identified in the Factor C enzyme of the LAL cascade that shows very high affinity for LPS. It has been used to remove endotoxin from water, buffers, and cell culture media. It has also been used to remove endotoxin from protein and DNA solutions with minimal product loss. Removal of endotoxin using **EndoBind-R™** is fast, easy and inexpensive.

EndoBind-R™

Catalog No: EBR-3005

www.solublebiotech.com

Removing Endotoxin from Protein Solutions

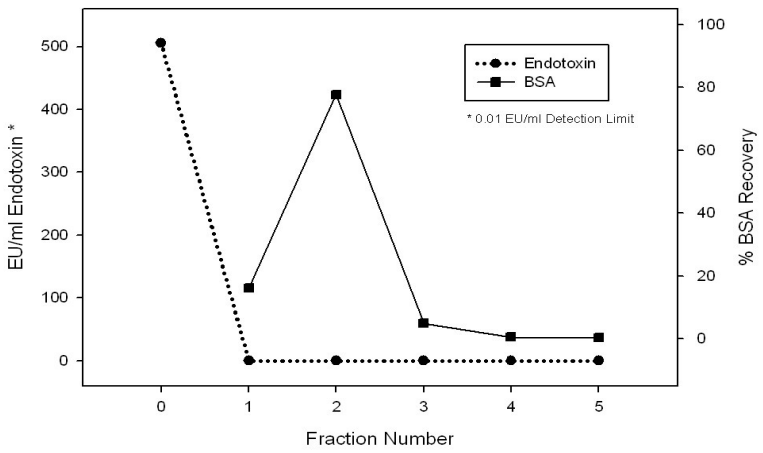

Figure 1. Endotoxin Removal from BSA. BSA samples at 1 mg/ml were prepared in 20 mM sodium acetate at pH 5.0 with 150 mM sodium chloride and 50 ng/ml *E. coli O55:B5* endotoxin and applied to EndoBind-R™. The protein was recovered in four subsequent 1 ml washes. Protein recovery was determined by absorbance and endotoxin levels were determined by PyroGene (Lonza) assay.

For optimal protein purification and recovery using **EndoBind-R™**, the pH and ionic strength of the buffer should be optimized in regard to the protein isoelectric point. As an example, bovine serum albumin (BSA) was purified. First, experiments showed that a 20 mM sodium acetate buffer at pH 5.0 containing 150 mM sodium chloride gave the best product recovery. This pH is slightly higher than the isoelectric point of BSA (4.6). Next, endotoxin removal from BSA at these conditions was measured. A 1 mg/ml solution of BSA was prepared in 20 mM sodium acetate at pH 5.0 containing 150 mM sodium chloride. The low endotoxin BSA was tested for contaminating endotoxin and found to be a rather low value of about 0.05 EU/mg. *E. coli O55:B5* endotoxin at a concentration of 50 ng/ml (500 EU/ml) was added to the protein solution and purified with **EndoBind-R™**. The flow-through, fraction 1, contained about 16% of the initial protein (Figure 1). However, the majority of BSA eluted into the first wash, fraction 2, as a 77% protein peak. Fractions 3 through 5 combined contained less than 6% of the initial BSA load. The LPS content in the sample load (fraction 0) measured 506 EU/ml and was reduced to below the detection limit of 0.01 EU/ml in all five column fractions. This represents more than 99.998 % LPS removal and over 99% protein recovery after purification with the **EndoBind-R™** column. Even the 0.05 EU/ml contaminating endotoxin was removed from the starting material.

For a more detailed explanation of buffer optimization and purification of protein solutions using **EndoBind-R™**, refer to the **Soluble Biotech, Inc. EndoBind-R™** Protein Purification Application Notes. This document outlines both salt and pH optimization protocols and their application to purify proteins such as bovine serum albumin, human transferrin, bovine liver catalase, hemoglobin from bovine erythrocytes, and rabbit IgG.

Removing Endotoxin from DNA Solutions

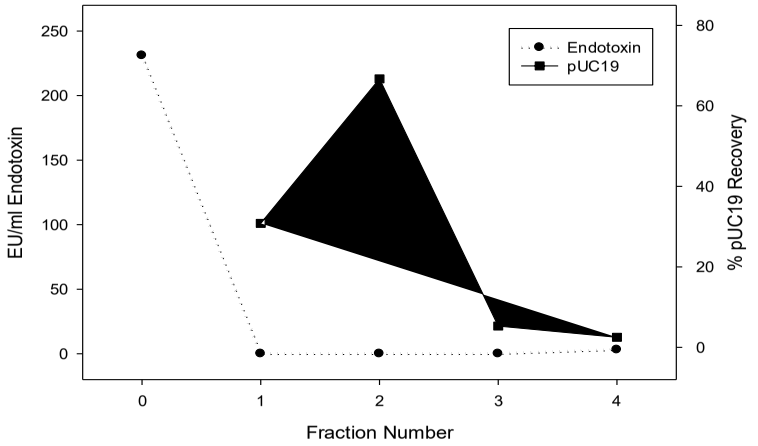

Figure 2. Endotoxin Removal from pUC19. pUC19 samples at 30 µg/ml were prepared in TE (10 mM Tris, 1 mM EDTA) at pH 8.0 with 1 M sodium chloride and 25 ng/ml *E. coli O55:B5* endotoxin and applied to EndoBind-R™. The DNA was recovered in three subsequent 1 ml washes. DNA recovery was determined by absorbance and endotoxin levels were determined by PyroGene (Lonza) assay.

DNA purification using **EndoBind-R™** was investigated using the common cloning vector pUC19. Previous experiments showed that a TE buffer at pH 8.0 containing 1 M sodium chloride was sufficient for high DNA recovery. To test endotoxin removal, a 30 µg/ml pUC19 solution was prepared in TE pH 8.0 with 1 M sodium chloride. *E. coli O55:B5* endotoxin was added to the solution at a concentration of 25 ng/ml (250 EU/ml) (fraction 0) and added to the **EndoBind-R™** column. The flow-through was collected as fraction 1. Next, the column was rinsed with three 1 ml washes of TE pH 8.0 with 1 M sodium chloride (fractions 2-4). DNA recovery was very high with about 30% of the initial load eluting in the flow-through and a peak value of nearly 67% in fraction 2 (Figure 2). In addition, endotoxin removal was nearly complete. The load contained 231 EU/ml (fraction 0) and was reduced to below the level of detection (0.01 EU/ml) in all samples collected from the **EndoBind-R™** column. This represents removal of over 99.99% of endotoxin with near complete product recovery. Similar experiments with small linear DNA fragments gave nearly identical results.

For a more detailed explanation of buffer optimization and purification of DNA solutions using **EndoBind-R™**, refer to the **Soluble Biottech, Inc. EndoBind-R™** DNA Purification Application Notes. This document outlines both salt and pH optimization protocols and their application to purify small, linear DNA fragments as well as plasmid samples with both high and low levels of endotoxin contamination.