



# ToxOut™ Phase Extraction Endotoxin Removal Kit

rev 05/20

(Cat # K2507-10; 10 Columns; Store at 4 °C, Do Not Freeze)

## I. Introduction:

Endotoxin is a lipopolysaccharide (LPS) complex located in the outer membrane of gram-negative bacteria. A single *E.coli* bacterium contains ~2 million LPS molecules. During experimental procedures, large amount of endotoxin is shed and can easily contaminate labware, buffers and downstream products. *In vitro*, endotoxin causes a variety of problems in cell-based research. *In vivo*, endotoxin may cause various side effects, including inflammatory response, organ failure or septic shock in host organisms. Therefore, it is critical to remove endotoxin from samples and products.

BioVision's ToxOut™ Phase Extraction Endotoxin Removal Kit can effectively eliminate high concentration endotoxin in solutions containing proteins or pharmacologically important components down to <0.05 EU/ml without using toxic buffers, while maintaining protein recovery at >90%. BioVision's ToxOut™ Phase Extraction Endotoxin Removal Kit is based on Triton X-114 Phase Extraction (PE), which shows strong endotoxin removal capabilities. The residual Triton X-114 is further eliminated with our proprietary beads to achieve minimal detergent level in the final samples. The protein recovery is >90% depending on individual protein and typically greater than 95%.

## II. Product Features:

- Lower endotoxin level to < 0.05 EU/ml in a single extraction from up to 5,000 EU/ml sample
- High Capacity: up to 99.99% endotoxin can be removed in a single extraction at 1E+7 EU/ml
- High Sample Recovery: >90% recovery with protein solution samples (typically > 95%)
- Very low residual detergent: < 1 µg/ml
- Simple and fast procedure: Takes less than one hour to get endotoxin-free sample
- One kit can clean up to 5 ml protein sample at 1-5 mg/ml of concentration
- Final products are suitable for potential downstream therapeutic applications as ligand and buffers are nontoxic

## III. Applications:

- Effectively eliminate endotoxins to < 0.05 EU/ml
- Ideal for processing small scale solution samples (0.1 ml - 0.5 ml)

## IV. Kit Contents:

Components	K2507-10	Part Number
ToxOut™ Phase Extraction Endotoxin Removal Reagent A	1.2 ml	K2507-10-1
ToxOut™ Phase Extraction Endotoxin Removal Reagent B	1.5 ml	K2507-10-2
ToxOut™ Phase Separation Indicator	25 µl	K2507-10-3
ToxOut™ Triton X-114 cleaning column	10 Columns	K2507-10-4
ToxOut™ Endotoxin-free Collection Tube	20 Tubes	K2507-10-5

## V. User Supplied Reagents and Equipment:

- Adjustable pipettes and sterile, endotoxin-free (or pyrogen-free) tips
- Centrifuge for 1.5 - 2 ml microcentrifuge and 1.5 ml conical tubes
- Each kit contains reagents and tubes sufficient for at least 20 extractions or 10 ml protein sample
- Additional endotoxin-free components are available at BioVision
  - ToxOut™ Endotoxin-free PBS buffer (Cat. No. 7943-50)
  - ToxOut™ Endotoxin-free Collection Tube (Cat. No. 7937-50)

## VI. Storage and Handling:

- Store kit and components at 4 °C. Do not freeze.
- Always use endotoxin-free solutions and tubes and proceed with extra caution.
- Read entire protocol before performing the experiment.

## VII. Endotoxin Removal Protocol:

### Notes:

- Keep the kit components at room temperature for 10-15 min before use.
- Mix reagent A by gentle upside down mixing several times before every use. Make sure the solution becomes cloudy. DO NOT VORTEX.
- Sample: Ideally protein sample should be in PBS or TBS at 1-5 mg/ml (max 10 mg/ml). It is recommended to change the buffer to PBS or TSB (not included). However, other buffers can be used and user might need to do smaller scale trial experiment.

### Procedure:

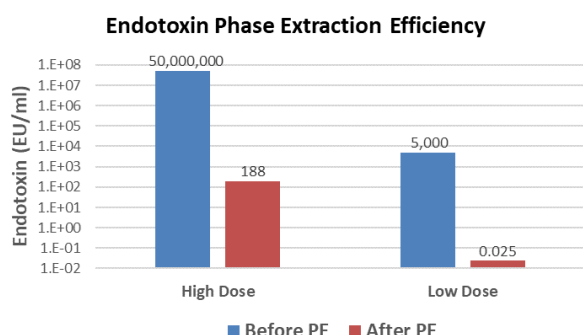
1. In a 1.5-ml conical Eppendorf tube (not included) add the following components:

Sample	500 µl
Reagent A	100 µl
Reagent B	120 µl
Indicator (optional)	1-2 µl
2. Incubate the solution at 4 °C for 5-10 min with gentle mixing. Make sure the solution becomes clear (not cloudy).

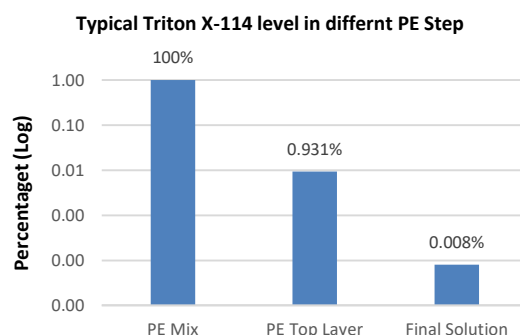
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- Place the sample tubes at 37 °C for 1-2 min (Optional: at room temperature for 5-10 minutes for unstable protein) with occasional gentle mixing. Make sure the solution becomes cloudy again.
- Spin the tube on desktop centrifuge at 14,000 rpm (16,000 x g) at room temperature for 5 min. The solution will be separated into two layers. The top layer is the endotoxin-free protein solution and the bottom layer is tinted TritonX-114. The bottom layer will be blue in color if the Indicator is added.
- Separately set Triton X-114 cleaning column on an Endotoxin-Free collection tube without opening the bottom stopper. Briefly spin at 3,000 rpm (700 x g) at room temperature. Remove the top screw cap and place it on clean endotoxin free surface/rack.
- Carefully aspirate the clear upper layer only from step 4 without disturbing or aspirating any of the tinted bottom Triton X-114 layer.
- Transfer the upper layer into the Triton X-114 cleaning column. Close the column with the top screw cap.
- Seal the column/tube assembly with parafilm (not included). Incubate the solution at 4 °C for 5-10 min with gentle mixing.
- Remove the parafilm, Snap off the bottom stopper and loose the cap slightly. (Put the column in a new Endotoxin-free Collection Tube if the tube was contaminated during incubation).
- Spin the column with the tube for 2 min at 3,000 rpm (700 x g) at room temperature.
- The collected centrifugate is the ET-Free sample.
- Determine endotoxin level and protein recovery.  
If the final endotoxin concentration is still above the desired endotoxin level, repeat the endotoxin removal procedure using new column (step 2 to step 11).



**Figure 1. ToxOut™ Phase Extraction Endotoxin Removal Kit Efficiency Tests.** **Left:** High dose Endotoxin removal tests; 500 µl of LPS/BSA solution at  $5 \times 10^7$  EU/ml LPS and 10 mg/ml BSA is used in the PE procedure and the endotoxin is reduced to 188 EU/ml. **Right:** Low dose Endotoxin removal tests; 500 µl of LPS/BSA solution at 5,000 EU/ml LPS and 10 mg/ml BSA is used in the PE procedure and the endotoxin is reduced to 0.025 EU/ml (endotoxin is reduced by >99.99% in both samples). Average protein recovery is > 95% (data not shown).



**Figure 2. The Residual Triton X-114 Level in different PE Steps.** The Triton X-114 PE Mix (500 µl) without protein was used in phase separation and top phase was purified on the column according to protocol. Triton X-114 concentration was determined by UV absorbance of the solutions with pure Triton X-114 solution used for the calibration curve.

#### VIII. Troubleshooting:

Problem	Cause	Solution
Low detox efficiency	• The pH of the sample is not between pH 6-8	• Adjust the sample to neutral pH (best range: pH 7-8)
	• Endotoxin concentration is too high in sample	• Dilute and Aliquot the sample to several extractions to avoid overloading endotoxin amount in one extraction • Repeat one or more extractions until endotoxin concentration reduces to desired value
	• External endotoxin contamination	• Use endotoxin-free solutions and lab ware
Low Protein Yield	• Phase separation is not completed	• Slightly adjust the Reagent B amount added. • Spin for longer time (step 4)
Too high Protein yield (>100%)	• Too much residue 'Triton X-114	• Extend the spin time • Separate the phase as soon as possible.
There is no phase separation	• Buffer condition is not good • Ion strength is too high or too low • There are other detergents in the sample	• Change buffer to PBS or TBS • Adjust the Reagent B usage • Remove other detergents

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**IX. Related Products:**

- ToxOut™ Rapid Endotoxin Removal Kit ( K2501)
- ToxOut™ Rapid Endotoxin Removal Agarose (7941)
- ToxOut™ Endotoxin Removal Equilibration Buffer (7940)
- ToxOut™ Endotoxin-free Water (7938)
- ToxOut™ Endotoxin-free PBS (7943)
- ToxOut™ Endotoxin-free Collection Tube, 2.0 ml (7937)
- ToxOut™ Endotoxin-free Glass Vial (7944)

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