



Endotoxin Process for Recombinant Protein Purification


Background

Endotoxins, or bacterial lipopolysaccharides (LPS), often contaminate recombinant protein preparations and are highly undesirable, especially when the protein prep is to be used in immunological assays. Presence of endotoxin can cause false readings in cell based assays; plus there are limits to the amounts of endotoxin allowed in human products.

LPS is hydrophobic due to lipid tails and has a net negative charge at slightly acidic, neutral and basic pH. These properties can be utilized when trying to remove endotoxin from protein. Many commercially available kits/methods for endotoxin removal are based on this principle; however, the results obtained vary greatly depending on the target protein and the amount of endotoxin present. Often, the protein binds to the matrix as efficiently as the endotoxin and cannot be recovered easily, or both endotoxin and the target protein co-elute in the flow-through fractions (others have had similar experiences).

Our Protein Sciences lab has optimized a method for endotoxin minimization, which relies on a methodology to control endotoxin contamination throughout the purification process. This method works very well to reduce typical endotoxin levels from >300,000 EU/ml to <10EU/ml.

Advantages

-  has been most extensively used and validated for endotoxin reduction for target recombinant proteins expressed in *E. coli*.
- The method works well for protein purified from the 'Soluble' fraction as well as from 'Refold' procedures.
- Significantly cost-effective as compared to endotoxin kits.
- Also works for proteins expressed in other systems, such as mammalian or insect cells.

Endotoxin References

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