RESEARCH CORPORATION TECHNOLOGIES

ClearColi™ EXPRESSION TECHNOLOGY

Endotoxin-Free ClearColi® BL21 (DE3) for Protein Expression: Reduced Risk of Masked Endotoxin

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Recently, the FDA outlined concerns about a newly described phenomenon known as Low Endotoxin Recovery (LER), where the industry standard limulus assay (LAL) is unable to detect even modest concentrations of lipopolysaccharide (LPS) (FDA, 2014). This phenomenon, which is also known as endotoxin masking, is thought to occur because of excipients interfering with LPS detection. Endotoxin, or LPS, is a major constituent of the outer membrane of nearly all gram-negative bacteria, such as *E. Coli.* LPS acts as a potent agonist of Toll-like receptor 4 (TLR4), eliciting pro-inflammatory activity in mammals, including humans. Even small doses of LPS can cause pyrogenic effects making the need for reliable methods to detect and remove endotoxin essential. Proteins manufactured in *E. coli* are generally contaminated with LPS, the removal of which is often difficult and costly. The LAL assay is the primary assay used by pharmaceutical companies for quality control of their therapeutic protein products. With the discovery of the phenomenon of masked endotoxin, there is a lack of a reliable validated assay to detect endotoxin in therapeutic proteins.

The major concern with masked endotoxin is that biologically meaningful levels of endotoxin, may remain undetected in manufactured therapeutic proteins creating a vulnerability for undetected contamination (Williams, 2015). Highlighting this concern, the FDA released a general guideline document in 2014 stating that adjuvant activity arising from microbial or host-cell-related contaminants, such as endotoxin in therapeutic protein products, may

cause an innate immune response (FDA, 2014). The immune response results in issues for therapeutic proteins:

- At low levels of endotoxin contamination, the endotoxin may act as an adjuvant, promoting the development of antibodies against the therapeutic protein. These antibodies may then bind to the therapeutic product reducing its efficacy.
- At higher levels of endotoxin contamination, the endotoxin may trigger pyrogenic and other serious adverse effects in patients.

Most Endotoxin contamination comes from the *E. coli* expression host. Therapeutic product contamination may result from multiple sources including outside contamination, poor removal of LPS, or the effect of masked endotoxin. The ClearColi® expression system eliminates endotoxin at the source, reducing the risk of endotoxin contamination in the final product.

What Benefit Does ClearColi® Offer to the Masked Endotoxin Issue?

To provide an alternative to endotoxin removal methods, ClearColi® cells were developed by genetically engineering *E. Coli* to have the endotoxin precursor lipid IV_A as the only LPS-related molecule in the outer membrane. This has been accomplished by the incorporation of seven genetic deletions (ΔgutQ, Δkds, Δ*lpxL*, Δ*lpxM*, Δ*pagP*, Δ*lpxP*, *and* Δ*eptA*) and a compensating mutation in msbA148. Lipid IV_A is not a TLR4 agonist and does not trigger an endotoxic response in humans. ClearColi® cells are viable and express proteins with comparable yields to other *E. coli* expression systems (Mamat 2015). In addition, lipid IV_A lacks the carbohydrate decorations usually attached to LPS allowing for easy downstream lipid IV_A removal and purification of therapeutic products.

A recent publication evaluated the potential for endotoxin masking of a recombinant protein expressed in either ClearColi® or an alternative commercially available *E. Coli* expression system (Schwarz et al., 2017). The proteins produced with both expression systems showed no signs of LPS contamination in the LAL assay. To confirm the lack of LPS contamination the recombinant proteins were further assessed with a human monocyte assay and a NF-κB reporter gene assay, both are highly sensitive methods to detect LPS contamination and can detect masked endotoxin. The ClearColi® expressed protein again showed no signs of LPS contamination in either the human monocyte assay or the NF-κB reporter gene assay. Meanwhile, the protein expressed in the alternative *E. Coli* expression system showed LPS contamination in both the human monocyte assay and the NF-κB reporter gene assay, likely resulting from endotoxin masking. The ClearColi® system as the expression host effectively reduced the risk of LPS contamination and masked endotoxin.

References

1. Chen J, Vinther A. Low endotoxin recovery in common biologics products. Presented at the PDA Annual Meeting, Orlando, FL. April 15-17, 2013.
2. "Guidance for Industry: Immunogenicity Assessment for Therapeutic Protein Products [excerpts]." *Biotechnology Law Report*32.3 (2013): 172-85. Web.
3. Mamat U, Wilke K, Bramhill D, Schromm AB, Lindner B, Kohl TA, Corchero JL, Villaverde A, Schaffer L, Head SR, Souvignier C, Meredith TC, Woodard RW. Detoxifying Escherichia coli for endotoxin-free production of recombinant proteins. Microb Cell Fact. 14: 57, 2015. Erratum in: Microb Cell Fact.;14:81, 2015.
4. Reich, Johannes, Pierre Lang, Holger Grallert, and Hubert Motschmann. "Masking of endotoxin in surfactant samples: Effects on Limulus-based detection systems." *Biologicals*44.5 (2016): 417-22. Web.
5. Schwarz, Harald, Jan Gornicec, Theresa Neuper, Maria Alejandra Parigiani, Michael Wallner, Albert Duschl, and Jutta Horejs-Hoeck. "Biological Activity of Masked Endotoxin." *Scientific Reports*7 (2017): 44750. Web.
6. Williams, Kevin. "Webinar: Low Endotoxin Recovery (LER) – Context and ..." *Lonza*. N.p., 21 October 2015. Web. 30 June 2017.
7. Wintzingerode, Friedrich Von. "Low Endotoxin Recovery, A Brief Overview." *American Pharmaceutical Review*. N.p., 30 Sept. 2016. Web. 30 June 2017.