Outstanding cGMP Enzymatic Technology in Purification

An Efficient Method to Remove Nucleic Acids in the Production of Biopharmaceuticals

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Nucleic acids, like DNA and RNA, are massively released to the fermentation broth when biopharmaceuticals—antibodies, vaccines, proteins, etc.—are produced. The viscosity of the media is then significantly enhanced, creating membrane fouling, and leading to a cumbersome and unpredictable downstream, particularly in steps depending on fluidity, such as filtrations, centrifugation, or chromatography. Furthermore, nucleic acids may adhere to particles like viruses or inclusion bodies, hampering the purification of such particles.

Importantly, safety regulations are strict for the biopharmaceuticals industry, and products to be marketed must be virtually free of nucleic acids (which are considered contaminants). Therefore, integrating nucleic acid removal is a challenging step, yet essential for the product quality and safety criteria during the process development phase.

To remove nucleic acids from biological samples, successful strategies have traditionally included chemical means, such as precipitation (using acid/base or solvent treatments), filtration (using adsorptive depth filters or tangential flow filtration), sonication, or chromatographic methods, among others. These technologies often require conditions that can lower the yield and purity of the desired products. To tackle that challenge, regulatory authorities recommend enzymatic steps to remove nucleic acids. In this context, unselective nucleases hydrolyse nucleic acids by cleaving the phosphodiester bonds, rendering small oligonucleotides of several base pairs under mild conditions.

To add value in this area, a genetically engineered form of Serratia marcescens endonuclease—called DENARASE®—has been recently launched by c-LEcta (Leipzig, Germany). The patented production microorganism is a Gram-positive *Bacillus sp*., which is known to be an endotoxin-free strain. Moreover, the employed fermentation media is free of animal-derived feedstocks and antibiotics, and therefore DENARASE is a BSE/TSE-free product, with a high viral safety (as no animal-derived materials are involved in any step of its production).

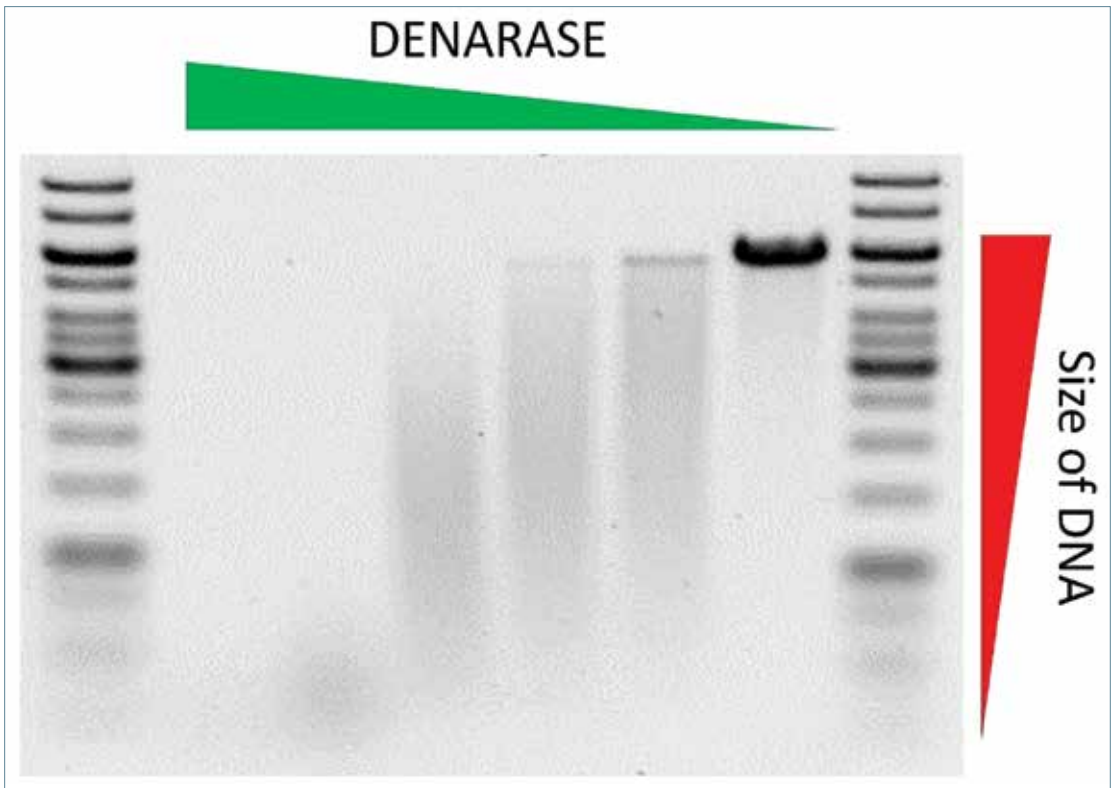

Figure. Use of DENARASE® to remove excess amounts of nucleic acids generated during the production of biopharmaceuticals.

Remarkably, DENARASE is now available at high quantities and purity, with an excellent quality-price ratio. This is due to the new production method, as *Bacillus sp*. secretes the enzyme extracellularly and is free of endotoxins. Both aspects significantly reduce costs in downstream processing. Notably, based on the production system, DENARASE manufacturing process is in full compliance with the cGMP requirements, something compulsory for red biotechnology.

The biochemical features of DENARASE are outstanding and enable its use in a broad array of processing conditions. Thus, DENARASE accepts a broad substrate range, cleaving all forms of nucleic acids, RNA and DNA, single- and double-stranded, as well as linear or circular sequences. The final products achieved are oligonucleotide fragments of 2–5 base pairs. Likewise, DENARASE is active along a wide operational frame of temperature (0–42 °C, optimum 37°C) and pH (5.5–10, optimum 8–9). Moreover, it remains active upon the addition of deleterious agents like ionic, non-ionic, or chaotropic agents, or denaturing compounds like urea, and accepts high ionic strengths of different buffers (up to 150 mM), or high concentrations of KCl or NaCl (typical salts of fermentation broths) of up to 300 mM.

Overall, these excellent features for DENARASE broaden the options for the nucleic acid removal and purification steps for the development of cell- and gene-therapy treatments, vaccines (e.g., AAV, lentiviruses, etc.), as well as many other applications in the bio/pharmaceutical industry, where the digestion/removal of nucleic acid residuals appears mandatory.

As stated above, a relevant application of DENARASE is the DNA/RNA removal from products obtained in fermentative processes aiming at producing biopharmaceuticals, enzymes, vaccines, or biological compounds following biosynthetic strategies. For the final manufacturing of these biopharmaceuticals, and to reach their ultimate commercialization, regulatory authorities restrict the nucleic acid levels to less than 100 pg per dose (applied to the end-product sample).

Once DENARASE has hydrolysed the nucleic acids *(Figure)*, its removal from the final product can be achieved by different chromatographic steps (namely, ionic exchange or hydrophobic interaction chromatography).

Likewise, if there are differences between the desired product and DENARASE, technologies like cross-flow filtration or depth filtration can be chosen as well. Therefore, already established purification steps of the product can be used for the removal of DENARASE, when the enzyme is used at an early stage of the whole process. The successful removal can be demonstrated by a commercially available DENARASE-ELISA kit, developed by c-LEcta, which is now available for that specific validation.

In summary, DENARASE can hydrolyse all types of nucleic acids, RNA or DNA, and displays a remarkably broad application range in the production of biopharmaceuticals. Under many different processing conditions, DENARASE keeps its unselective and excellent hydrolytic performance.

DENARASE is based on the recombinant expression in an endotoxin-free Gram-positive *Bacillus sp*. strain as microbial host, rendering a high purity of >99% at competitive prices. The fermentation procedure does not use animal-derived feedstocks, conferring DENARASE the consideration of BSE/TSE-free product, with a high viral safety. This uniqueness, in full compliance with the cGMP requirements, is enabling DENARASE to generate added value to biotechnological and biopharmaceutical applications, like nucleic acid removal, biosynthesis, vaccine production, cell-therapy, oncology, CAR-T cell development, etc.

Beyond biopharmaceuticals, for food and feed products, there is another new derivative available at c-LEcta: The so-called NuCLEANase food-grade is a nuclease which is produced according to food and feed standards, being kosher- and halal-certified. NuCLEANase is yet another excellent alternative of high quality and efficiency, accessible at competitive price.

Interested? DENARASE can be ordered directly from c-LEcta (www.c-lecta.com), or within Europe, through its new distribution partner, VWR (www.vwr.com), a part of Avantor. The supply of DENARASE is always secured, as c-LEcta keeps up to 100 MU of enzyme on permanent stock for its customers. Importantly, users of other nucleases in the bio/pharma industry should assess DENARASE in their processes as well, to have it validated as a high quality second source of nuclease, just in case of breakage of stock of their main suppliers. GEN

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