Practical Guide: Selecting the Optimal Resins for Removal of DNA Contamination during Process Purification

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Purification Solutions

Bulletin 6881

Effectively Eliminate DNA Contamination with Our Versatile Process Resins

Cell culture production of recombinant proteins invariably results in a protein sample contaminated with host cell DNA. The contaminating DNA leads to increased viscosity of the feedstream and can interfere with subsequent purification steps such as anion exchange chromatography. In addition, contamination with cellular DNA creates a therapeutic risk. Regulatory authorities require that DNA levels in all therapeutic protein and antibody samples be reduced to 10–100 pg/dose. Cell culture clarification processes, such as centrifugation or tangential flow filtration (TFF), can provide some initial DNA removal. However, such techniques create high-shear conditions, which could increase cell disruption and, as a result, contamination. In addition, use of Benzonase or other nucleases can lead to contamination with DNA fragments instead of the full-length DNA and requires subsequent nuclease removal. Moreover, this method does not maintain the DNA binding activity of some proteins, which can be a requirement of the purification process. In contrast, chromatography avoids these issues, resulting in improved DNA removal from process streams.

Bio-Rad has provided a progressive selection of chromatography resins for the process-scale purification of proteins for more than 50 years. This guide highlights resins that can be used for the removal of DNA contamination during protein purification (bulletin 6810), monoclonal antibody (mAb) purification (bulletin 6875) and IgG purification (bulletin 6793).

Affinity Chromatography Resin

UNOsphere SUPrA™ Resin

This resin is designed for process-scale purification of monoclonal and polyclonal antibodies. Higher bed heights can be used to increase residence time without excessive pressure increases, providing a large window of operational freedom. Purification with a Protein A–based affinity resin is often the first step when purifying feedstream since it can lead to >90% removal of DNA and other contaminants, such as host cell proteins (HCP), with minimal buffer optimization. UNOsphere SUPrA has been shown to significantly decrease DNA contamination levels during antibody purification (Table 1) (bulletin 5728).

Table 1. DNA clearance from cell extracts using different resins.

Purification Step	Resin	DNA Content, ng/mg mAb1	DNA Content, ng/mg mAb2
Cell culture supernatant	–	>5 x 10^3	>1.6 x 10^5
Capture	UNOsphere SUPrA	18.6	19
Intermediate	UNOsphere™ Q	3.5	1.9
Polish	CHT™ Ceramic Hydroxyapatite	2.5	3

Ion Exchange (IEX) Chromatography Resins

Since DNA is typically negatively charged, IEX resins can be used to separate it from target biomolecules of similar or opposite charge using appropriate buffer conditions.

Cation Exchange (CEX) Resins

Nuvia™ S Resin

Nuvia S Resin is an ultra high capacity next-generation CEX resin built on the industry-proven UNOsphere base matrix technology. It provides very high capture and exceptional flow properties designed to meet current and future process needs. It has readily available negatively charged groups for biomolecule binding. Host cell DNA does not adsorb to this matrix under normal circumstances since both the negative charges repel each other. Therefore, DNA comes out in the flowthrough and the initial column washes, while the target protein binds to the resin. Consequently, bind/elute mode can remove more than three logs of DNA from the sample as shown in Table 2 (bulletin 6241).

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Anion Exchange (AEX) Resins

Negatively charged DNA binds to the positive charges on these ligands and is retained on the column while the target biomolecule ends up in the flowthrough. AEX resins are typically used in intermediate purification steps after the feedstream has gone through an initial round of capture purification. AEX resins should not be used in capture steps since they could become saturated with bound DNA, which would lead to decreased ionic binding capacity for other contaminants.

Nuvia Q Resin

Nuvia Q Resin is an ultra high capacity next-generation AEX resin. It delivers high binding capacity over a range of pH and flow rates. It can effectively clear DNA contamination from a CHO cell culture harvest when used in the intermediate step during mAb purification (Table 2).

Table 2. DNA clearance from CHO cell extract with different resins.

Purification Step	Resin	DNA Content, ng/mg
Cell culture supernatant	–	9.3×10^4
Capture	Nuvia S	17
Intermediate	Nuvia Q	4.1
Polish	Nuvia™ cPrime™	<0.008

UNOsphere Q Resin

UNOsphere Q is a high-capacity high-throughput AEX resin based on acrylamido and vinylic monomers, designed for process chromatography. The resin was designed with large-diameter pores and a large surface area to maximize capture speed, macromolecule capacity, recovery, and productivity. It follows the same principle for removal of host cell dsDNA as Nuvia Q. It can reduce DNA levels by multiple logs during mAb purification when used in the intermediate purification step (Table 1) (bulletin 5728).

Mixed-Mode Resins/Media

Mixed-mode resins/media combine the functionality of two or more unimodal resins. As a result of their multifunctionality, the resins can interact with biomolecules through multiple modes, making it possible to achieve greater purity while decreasing the number of purification steps. These resins offer an orthogonal approach to purification by IEX or hydrophobic interaction chromatography (HIC) for difficult-to-purify biomolecules.

CHT Ceramic Hydroxyapatite Media

CHT has unique separation properties and unparalleled selectivity and resolution. It often separates proteins shown to be homogeneous by electrophoresis and chromatographic techniques. Hydroxyapatite contains two types of binding sites — positively charged calcium and negatively charged phosphate groups. Solute species dominantly interact through CEX via the phosphate groups and/or through metal affinity via the calcium atoms. Although DNA molecules are repelled by the phosphate groups, they are very tightly bound via multiple metal affinity interactions to the positively charged Ca^{2+} groups. CHT provides significant (often >4 log) clearance of DNA contamination levels during antibody purification (bulletin RP0033).

Nuvia cPrime Resin

Nuvia cPrime Resin is designed with a mixed-mode ligand that provides a unique balance between hydrophobic and charged characteristics. It is effective for the purification of established therapeutic proteins as well as the increasingly diverse new constructs that are in development, many of which lack an affinity handle. It has been shown to be very effective in clearing DNA contamination from a CHO cell culture harvest for purification of a mAb when used in the final polish step (Table 2) (bulletin 6241).

The details provided here can help you design a process purification strategy for removal of DNA contamination. For technical/product support or to request a quote, email your regional Bio-Rad representative at **process@bio-rad.com** or contact customer service at 1-800-4-BIORAD (1-800-424-6723).

Explore our extensive selection of process-scale chromatography resins and their performance characteristics and applications (bulletin 6713). For process optimization of your purification workflow, request a sample.

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