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Technical Bulletin

Protein Purification Techniques
Vol. 3. Nucleic Acid Precipitation, Proteolysis Inhibition and Lipid Removal

Introduction
Nucleic acids, lipids and proteases can often be in high concentrations in cell culture supernatants, contaminating the target protein. In addition, the method of extraction may increase the presence of these contaminants. Measures need to be taken to limit the effect of these contaminants on the downstream purification process.

Nucleic Acid Removal
Steps to remove nucleic acids include the addition of protamine sulfate, streptomycin, magnesium chloride, spermine, polyethylenaimine (PEI) or the appropriate nucleases, although nucleases should be avoid if possible.[1,2] In addition, the use of positively charged anion-exchange resins can remove nucleic acids but may be less efficient for large-scale work.[3] Although the resins may absorb less target protein than other techniques, they are typically required in large amounts to be effective.

In addition, precipitation of proteins away from nucleic acids is possible with dextran-polyethylene glycol and high concentrations of sodium chloride.[4] Care should be taken when removing nucleic acids to avoid the proteolytic damage or removal of the target protein. It is highly recommended that all protein purification steps take place at 2 to 8 C whenever possible to avoid damage by proteases.

Protease Inhibitors
Typically, proteases are inactivated or inhibited by the addition of any number of chemicals. For example: PMSF (phenylmethylsulfonyl fluoride), benzamidine and aprotinin are for serine proteases; leupeptin and antipain for thiol proteases; ethylenediaminetetraacetic acid (EDTA) and ethylene glycol-bis(β-aminoethyl ether) N, N, N′, N′,-tetraacetic acid (EGTA) for metalloproteases; and pepstatin A for acid proteases.[2,3] A combination of protease inhibitors, most commonly EDTA and PMSF, is recommended. Typical working concentrations are 0.1 mM - 1 mM.

Care should be taken when using protease inhibitors, as they can be unstable and toxic under certain circumstances. Typically, proteases require pre-solubilization in organic solvents such as iso-propanol, ethanol or dimethyl sulfoxide (DMSO). Once in aqueous solutions, it is very common for protease inhibitors to rapidly hydrolyze. Therefore, it is often necessary to add additional inhibitors throughout the purification sequence.

Lipid and Surfactant Removal
Another interfering component common to supernatants from cell culture are lipids. Some lipids can be classified as detergents, also known as surfactants. Surfactants aid in the solubility of a protein or metabolic component and their absorption into the cells. Although lipids and surfactants are often necessary components in cell culture media, they can present difficulties during downstream purification. Surfactants often coat proteins and therefore can limit their accessibility to purification steps, in particular, chromatography. Therefore, it may be necessary to remove lipids and surfactants prior to many purification steps.

The removal of lipids and surfactants from the protein mixture can be accomplished several ways, some of which are: trichloroacetic acid (TCA) to precipitate detergents;[2] SM-2 macroporous beads, batch or column chromatography to remove proteins from surfactants and lipids; hydroxyapatite coated ceramic beads, batch or column chromatography to remove proteins from surfactants and lipids; and polyethylene glycol (PEG) to precipitate the proteins, leaving lipids and surfactants in the supernatant.

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Another method to remove surfactants that may work under limited circumstances utilizes the surfactant's cloud point. At the cloud point, a surfactant solution passes from an isotropic, micellar system to a two-phased system.[3] One phase is depleted in surfactant and the other is rich in giant micelles. The disadvantage of this method is the requirement of cooling the cloudy solution until the 2 layers form. In addition, this effect may only occur when the surfactants are used at high concentrations.

Nucleic acid precipitation, proteolysis inhibition and lipid removal require a great deal of optimization. For more information about this subject or other SAFC Biosciences' products and services, please contact our Technical Services department.

References

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