DISSOLVING BSA IN SOLUTION

Dissolving freeze-dried Bovine Serum Albumin (BSA) powders can sometimes be complicated. BSA is a highly soluble protein due to its strong negative charge and affinity for water, but getting the powder into solution quickly, easily, and with minimal foaming is dependent on a number of factors. We have compiled some information and a list of common questions below to facilitate dissolving BSA.

THE SCIENCE BEHIND BSA SOLUBILIZATION

Before determining the best BSA reconstitution method, it's important to understand some of the science behind albumin protein. The albumin molecule, like every protein, gets its unique globular shape from the folding of amino acids. The secondary structure of amino acids that form the albumin protein have areas that are both hydrophilic (have a high affinity for water) and hydrophobic (repels from water) which interact differently with the solution (Masuelli, 2013). BSA that has been enhanced with exogenous lipids tends to take longer to suspend. The reason for this is because the lipids bind to the hydrophilic sites on the molecule which prevents ions in solution from associating with those sites, thus reducing the protein's overall interaction with water. Similarly, polymerized albumin molecules bind to other molecules through hydrophilic sites, preventing more frequent interactions with the solution.

WHAT IS THE BEST STEP BY STEP PROCESS FOR DISSOLVING BSA?

Slowly add BSA powder to the water or solvent with slow mixing. Mixing should be controlled to draw the powder down into the liquid, not build up on the surface, and not create excess foam. The rate of addition should allow the powder to go into solution and avoiding excess clumping of the powder. For example, start by slowly adding 1% (weight BSA/ final weight of solution) BSA powder as described above. Wait until the first 1% of powder is in solution before adding the next 1%. Slow the rate of BSA powder addition as the protein concentration gets higher. To enhance solubilization, especially for a 30% solution, allow the solution to reach room temperature (20 °C) while mixing. Once all the powder is in solution, slow down the mixing to remove any entrained air.

WHAT IS THE BEST WAY TO PREVENT A SOLUTION FROM FOAMING?

It is not uncommon to see foaming when making a BSA solution which can often lead to longer suspension times. Foam can be caused by BSA's polymeric and polyelectrolyte nature which causes repulsions, or bubbles, during the absorption process (Krzan, Caps and Vandewalle, 2013.) To avoid foaming, rock or swirl the solution slowly. If agitation is insufficient, mix the solution gently by using stir bars or agitators at the slowest speeds that pull the powder down into solution. Foam can be problematic because foam bubbles can trap small lumps of BSA that will increase the non-specific binding in the solution.

WHAT IS THE BEST METHOD FOR DISSOLVING LARGE BATCHES OF BSA?

Large batches should be stirred on the lowest setting of the mixing equipment you are using; it may take multiple days to fully suspend BSA in a large batch. Additionally, if you are adding salts to your solution, adding the salts prior to the BSA can help the polar portions of the protein disassociate, creating more interactions and therefore allowing it to be more soluble.

HOW SHOULD A PREPARED SOLUTION BE STORED?

Although BSA prepared in an area with low contamination can be stored at room temperature, we recommend that once your BSA solution is prepared, to store it at 2-8° C. We also recommend filtering the solution prior to storage to reduce the potential for bacterial contamination.

WHAT IS THE BEST SOLUTION TO SUSPEND BSA IN?

Solvents are application based, but typically salt buffers or pure water solutions will work the best for most applications. Typically, albumin close to a neutral pH is soluble up to 35% in a salt solution and up to 50% in a pure water solution.

WHAT IS THE BEST FILTER TO USE ONCE A BSA STOCK SOLUTION IS MADE?

A 0.2 µm filter is the best choice to reduce the potential for any bacterial contamination. With this filter size, a dilute solution should filter well. At high concentrations a filtration train is typically required to sterile filter the solution. A set of depth filters, with the first having a nominal pore size in the 0.5 to 1.0 µm range followed by another with a maximum nominal pore size of 0.5 µm, serve to protect a final sterilizing 0.2 µm membrane filter. The type of depth filter and membrane filter used is application specific, but polyethersulfone (PES), cellulose acetate (CA), and polyvinylidene fluoride (PVDF) membranes are typically used for protein filtration due to their low protein binding characteristic.

For technical questions and more, don't hesitate to contact our team to see how we can help you.

REFERENCES

Krzan, M., Caps, H., & Vandewalle, N.(2013). High stability of the bovine serum albumin foams evidence in Hele Shaw cell. Colloids and Surfaces A: Physicochemica/ and Engineering Aspects, 438, 112-118.

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