

Technically Speaking

BSA and Restriction Enzyme Digestions

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Restriction enzymes are used in a wide array of applications and reaction conditions. Many restriction enzymes show enhanced activity with the addition of BSA to the digestion reaction. BSA is provided with all Promega's restriction enzymes, and we recommend the addition of BSA to all restriction enzyme digestion reactions.

Q: What is BSA and why is the acetylated form used with restriction enzymes?

BSA, or bovine serum albumin, is a 66.2kDa protein which is commonly used as a "carrier" or nonspecific competitor (e.g., of proteases and adsorbents) in enzyme storage, dilution and assay buffers. All of the BSA used in Promega's assays and products is acetylated using acetic anhydride and is extensively tested to ensure that it is free of contaminating activities. The process of acetylation inactivates any nucleases that may otherwise complicate reactions involving nucleic acids. In this article, BSA always refers to acetylated bovine serum albumin.

Q: Is BSA necessary for restriction enzyme digests?

Although it is not necessary, the addition of BSA to restriction enzyme digests can result in an increase in the apparent "activity" of a restriction enzyme. The extent of this enhancement varies depending on the restriction enzyme involved (1). While there are a few enzymes for which the increase in activity observed as a result of the addition of BSA may be negligible, there are no known cases in which BSA is harmful to a restriction enzyme digest. The importance of BSA appears to be a function of DNA purity, the size and scale of the reaction, reaction vessel quality and material, and individual enzyme characteristics, as well as other factors not yet elucidated.

Q: How concentrated is the BSA solution provided with Promega's restriction enzymes?

BSA is provided at a concentration of 10mg/ml. This is a 100X solution, which means that it must be diluted 1:100 to achieve the recommended working concentration of 0.1mg/ml or 0.1µg/µl (1X) in each digestion reaction.

Q: What does it mean for a reagent to be used at a 1X concentration?

The assay buffers which accompany Promega's restriction enzymes are provided at a concentration of 10X, which means that the buffer should be diluted into an assay (i.e., digestion reaction) at a 1:10 ratio. This means that for every one microliter of buffer there should be nine microliters of other ingredients added to the assay for a reaction with a final volume of 10 microliters.

Q: What if I use too much BSA?

We have tested several enzymes using up to 1mg/ml BSA (ten times the recommended concentration) and have found no adverse effects on activity.

Q: Is it important to add BSA to enzyme dilutions, just the reaction mixture or both?

Many proteins, including restriction enzymes, are most stable at higher concentrations, and therefore are packaged that way. Due to the "stickiness" of some proteins, as well as other issues, it is usually wise to include an appropriately qualified BSA in the dilution buffer before adding the protein of interest. Restriction enzymes are usually stored in a buffer containing BSA and, when they are diluted, should also be added to an appropriate buffer containing BSA. When performing multiple digests it may be expedient to create a "pre-mix" containing water, buffer (final concentration approximately 1X), BSA and the appropriate restriction enzyme (with proper mixing before addition of the enzyme). This pre-mix solution is then divided among several tubes and different DNA constructs are added to each tube.

Q: What choice do I have for methods to incorporate BSA into restriction enzyme digests?

BSA is provided in a separate tube in order to accommodate individual preferences for using (or not using) BSA in reactions and to permit variation in how one wishes to use it. The 100X BSA stock solution may be used for daily restriction enzyme work by either diluting all or some of the BSA in the master tube to a 10X "working" stock. Alternatively, the 100X stock may be added directly to the 10X assay buffer or to a reaction pre-mix. Addition of BSA to a 10X reaction buffer will produce a 9X assay buffer containing BSA. We do not recommend the addition of BSA to 10X assay buffers stored at 4°C due to the increased risk of microbial growth as a result of nonsterile handling during use.

Q: Will BSA in a restriction enzyme digest affect downstream applications?

Most routine molecular biology applications, such as ligations, will not be affected adversely by carryover of BSA from a restriction enzyme digest. Rather, it is likely that residual BSA will support other enzyme reactions just as it does during restriction enzyme assays. If necessary, BSA may be removed by various methods including organic extraction or by using the Wizard[®] DNA Clean-Up System (Cat.# A7280).

Q: Should BSA be used with all restriction enzymes from any manufacturer?

BSA is traditionally used with many enzymes, including restriction enzymes. Many manufacturers either offer BSA for sale in conjunction with enzymes in general, or include it with the restriction enzyme product.

Reference

1. Williams, R. *et al.* (1996) *Promega Notes* **59**, 46.
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