Cell Culture Notes

Should I Heat Inactivate My Serum ?

Heat Inactivation of Serum

Routine Heat Inactivation of Animal Serum is performed in many tissue culture labs as part of the normal lab procedures. However, in many cases it is a procedure that does not have any benefit. Instead it can adversely effect the quality and performance of your serum.

Heat inactivation of serum has been an established laboratory procedure since before the 1970s. It was originally introduced for the purpose of inactivating serum complement proteins present in sera from animals bled after the neo-natal period (i.e. calf or adult sera). For serum obtained from calves or adults, Heat Inactivation is a proven method for reducing or eliminating serum complement activity that otherwise might be detrimental to the culture conditions. Nevertheless, most cells grow quite well in non fetal serum that has not been heat inactivated.

Fetal Bovine Serum, on the other hand, has been shown to have much reduced, or even non-existent levels of various serum complement factors (1). Consequently, groups seeking to inactivate serum complement in their fetal serum by employing a heat inactivation step are not getting any benefit from the task. Instead, they are subjecting their costly fetal serum to a heat stress that is known to inactivate proteins, peptides, vitamins, amino acids, and others co-factors, possibly damaging many beneficial components in the serum.

What about mycoplasma control ? When serum was first commercially available, it was filtered through a 0.45 µm sterile filters. At that time it was believed that Heat Inactivation would reduce the possibility of mycoplasma contamination arising from the serum. With the introduction of multiple 0.1 µm filtration of sera, continued improvement and validation of filtration systems, as well as testing and reporting for presence of mycoplasma, the need to rely on Heat Inactivation to reduce the risk of mycoplasma from serum has been eliminated.

Claims have been made that Heat Inactivation may reduce endogenous bovine virus contamination in the serum, but data showing complete removal of virus has not been reported. Thus, relying on Heat Inactivation for virus control is questionable and may not be reliable.

Since there does not appear to be many benefits to Heat Inactivation of Serum (especially for Fetal Serum), are there definite drawbacks ? HyClone has published a controlled study of 11 cell lines that were cultured with serum that was either Heat Inactivated (56EC 30 min, with intermittent swirling) or untreated (2). Six of 11 lines showed adverse growth with the treated serum; 3 lines showed no change, and 2 lines showed slightly improved growth as a result of heat inactivation. So, heat inactivation of fetal bovine serum, even when done properly, resulted in reduced growth or no change in the majority of cell lines.

In this same study, the effect of heating serum to a higher temperature was evaluated. Some Heat Inactivation protocols call for heating serum to 65EC for 30 minutes. It was found that heating fetal serum to 65EC for 30 minutes resulted in drastically reduced growth performance. Similarly, extended exposure of serum to a 56EC water bath resulted in reduced serum performance, and increased precipitate formation. (Side Note: While precipitate formation is not a cosmetically attractive feature, it is, in itself, not necessarily detrimental to serum performance. Precipitate formation can occur upon thawing, without exposure to higher temperatures. However, once precipitates start to form, exposure to elevated temperatures usually results in more precipitate forming. This can be problematic, as a user unfamiliar with the phenomenon might conclude that they have a bacterial contamination.).

Keep in mind that these negative effects occurred in a controlled study employing strict adherence to the heat inactivation protocol. The negative effect of Heat Inactivation can be compounded by other variables that a typical tissue culture lab encounters. Variables such as the depth of water in the water bath, whether glass or PETG bottles are used, or how the serum is cooled can effect the length of time serum components are exposed to potentially damaging heat, and thus effect performance.

To summarize, most cell culture groups do not need to heat inactivate their serum, fetal or otherwise. The procedure generally does not offer a benefit that warrants the time and effort to perform. The fact may be that the procedure does more harm to the performance of the serum than realized. Groups that do their own heat inactivation, or who purchase their serum already heat-inactivated, should consider carefully why they do so. If there is a question, they should determine empirically if Heat Inactivation is providing benefit to their tissue culture, or merely adding unnecessary work and risk.

References

1. Triglia, R.P., Linscott, W.D. 1980. Titers of nine complement components, conglutinin and C3b inactivator in adult and fetal bovine sera. Molecular Immunology 17:741-748.
2. HyClone 1996. Art to Science Vol. 15/ 1: 1-5.

Heat Inactivation Protocol for Serum

1. Remove Serum from freezer and allow bottles to acclimate to RT for 10 minutes.
2. Prepare a Control Bottle containing an equivalent volume of water as the serum. The bottle must be the same size and material as the serum bottle. Suspend a thermometer in the bottle without touching the sides or bottom of the bottle.
3. Transfer the serum and control bottles into a 37EC water bath. Fill the bath to just above the level of the serum. DO NOT immerse the caps.
4. Gently swirl the bottles every 10 minutes until thawed. Frequent agitation is very important while the serum thaws, as this disrupts concentration gradients of salts and proteins that can lead to precipitates forming.
5. Transfer the completely thawed and warmed bottles of serum and the control bottle into a water bath pre-heated to 56EC. Again, the bath should have enough water to rise above the level of the serum, but not touch the caps.
6. Swirl the bottles every 10 minutes while warming. Closely watch the thermometer in the Control Bottle. As soon as the temperature in the Control Bottle has reached 56EC, begin timing the inactivation.
7. Continue to mix every 10 minutes. Monitor the temperature of the bath and the control bottle.
8. At the end of the 30 minute inactivation, place bottles in an ice water bath for 15 minutes. Label and store at 4EC, or re-freeze at - 10EC to - 40EC.

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