TECHNICAL BULLETIN
Heat Inactivation of Serum

All of Atlanta Biologicals' animal sera are also available heat inactivated.

The objective of heat inactivation is to destroy complement activity in the serum without affecting the growth-promoting characteristics of the product. Removal of complement activity from the serum is not required for most cell cultures, but may be necessary for cultures that are sensitive to the complement activity. Since heat inactivation of the serum may, to some extent, decrease the growth performance properties of the serum, this procedure should only be performed if actually required for optimal cell growth. Researchers should evaluate the applicability of heat inactivation in regards to their own application.

If heat inactivation is required, the process should be carefully controlled to avoid increased formation of crystalline and flocculent precipitates, gelling of serum proteins and excessive loss of growth performance. Significant damage to serum can occur when it is subjected to higher than required temperatures or heated over extended lengths of time.

Follow the steps given below and do not leave the serum in the heated water bath for longer than necessary. Monitor the process to avoid excessive inactivation.

Heat Inactivation Protocol

- Thaw the serum following the guidelines outlined in "Storing, Thawing and Freezing Serum" and mix the contents of the bottle thoroughly.
- Heat a circulating water bath to 56 ^{o}C. Make sure that the bath is large enough to accommodate and immerse the number of bottles being inactivated.
- Place the thawed serum bottles in the water bath so that each bottle is completely immersed up to the level of the serum. Do not immerse the cap.
- Monitor the temperature in the water bath. When the temperature of the water bath returns to 56^{o}C, start timing the process for 30 minutes. Agitate the bottles approximately every 5 minutes during the heat inactivation process to prevent gelling of the serum proteins and to promote more uniform heating of the serum.
- After 30 minutes of heat inactivation at 56^{o}C, remove the serum bottles from the water bath and rapidly cool them in an ice bath. Prolonged treatment of the serum at elevated temperatures will cause deterioration of serum components critical for growth of cells.

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A Note Concerning Precipitates & Flocculent Material

Serum that has been frozen and thawed, or heat inactivated may contain some turbidity, flocculent material or crystalline precipitates. This is a normal occurrence with serum products and in no way indicates that the quality of the product has been compromised.

Frequently, this material is composed of fibrin that has converted from the soluble precursor form, fibrinogen, in serum. We collect and process our sera rapidly at cold temperatures to yield the highest quality serum with excellent growth properties. This rapid cold processing allows some soluble fibrinogen to remain in the serum after filtration which may convert to fibrin upon thawing.

Precipitates found in serum also frequently contain calcium complexes of inorganic serum components and proteins. Lipid serum components may also cause turbidity of the serum product. Incorrect thawing, frequent thaw-freeze cycles, heat inactivation and extended storage at temperatures above freezing will result in a greater amount of precipitates.

The presence of these substances in serum does not alter the performance characteristics of the product when used as a growth supplement for cell culture. It is not recommended to filter the serum to remove these precipitates. Doing so may result in the loss of some serum nutrients.

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