Heat Inactivation of Serum Products

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 serum, such as fetal bovine 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 required for optimal cell growth. Researchers should evaluate the applicability of heat inactivation regarding their own application. If you prefer, all serum products from R&D Systems are also available in heat inactivated forms:

View All Heat Inactivated Fetal Bovine Serum (FBS) Products

View All Other Heat Inactivated Serum Products

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.

Equipment:

- Circulating hot water bath, suitable size for the number of bottles to be heat inactivated
- Timer
- Thermometer
- Ice bath

Preparation:

1. Heat circulating water bath to a temperature of 56 °C. Make sure the water bath is large enough to accommodate and immerse the number of bottles being inactivated
2. Thaw serum following the protocol, “Storing, Thawing, and Freezing Serum” [Link to other protocol] and mix the contents of the bottle thoroughly before beginning to heat inactivate the serum

Protocol for Heat Inactivation

1. Place the thawed serum bottles in the water bath so that each bottle is completely immersed up to the level of the serum in the bottle. Do not immerse the cap.
2. Monitor the temperature in the water bath until it returns to 56 °C.
3. When the temperature of the water bath returns to 56 °C after the addition of the thawed serum bottles, set a timer for 30 minutes for the heat inactivation to proceed.
4. Agitate the bottles approximately every 5 minutes during the heat inactivation process.

   Note: *Agitation prevents gelling of the serum proteins and to promote more uniform heating of the serum.*
5. After 30 minutes of heat inactivation at 56 °C, remove the serum bottles from the water bath and transfer into ice bath for rapid cooling of the product to complete the heat inactivation process.

   Note: *Prolonged treatment of the serum at elevated temperatures will cause deterioration of serum components critical for cell growth.*

Protocol Note: Precipitates & Flocculent Material in Serum Products

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. At R&D Systems, 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 after filtration which may convert to fibrin upon thawing the serum.

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 precipitate.

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.