

Heat Inactivation of Serum

Serum is a very complex supplement containing mostly proteins, but also growth factors, hormones, amino acids, glucose, trypsin inhibitors, and lipids. The most common types of sera include: fetal bovine (FBS), newborn calf (NCS), horse (equine), pig (porcine), and human. There are also several different forms of serum subtypes defined by the types of processing available. Fetal bovine serum has been adopted as the standard supplement because of its rich content of growth factors and its low gamma globulin content.

The major functions of serum are to provide basic nutrients, hormones and growth factors, attachment and spreading factors, pH buffer, protease inhibitors, binding proteins carrying hormones, vitamins, minerals, and lipids. Serum also protects

against toxic effects associated with pH change, the presence of heavy metals ions, endotoxin, and proteolytic activity.

Since serum is a blood product it contains complement which can lead to complement-mediated cell lysis. To eliminate this risk, serum can be heat-inactivated. Heat inactivation destroys the complement, but can also, in some instances, destroy heat-labile growth factors, vitamins, amino acids, and hormones present in serum. Damage to these from extended heating or from temperatures greater than 56°C will decrease cell growth. Heating often causes the proteins and lipids concentrated at the bottom of the bottle to appear as white globules. Constant swirling during the heat inactivation process will help solve this problem.

The following is a suggested procedure for proper heat-inactivation of serum.

Procedure

1. Allow frozen serum to thaw under refrigerated conditions, at room temperature, or in a water bath. Serum can be thawed in a 37°C water bath, it must be removed from the water bath as soon as the serum is thawed.
2. To start heat inactivation process, prepare the water bath. Adjust the temperature of a water bath to 56°C and allow a thermometer to equilibrate to the temperature of the water. The thermometer should be placed in a bottle of water bath, where the volume of the bottle of serum will sit.
3. Once the serum has thawed completely, mix it by gently swirling the container to ensure that the solution is homogenous. Transfer to the water bath. Do not submerge the bottles or allow the water level to extend to the caps of the bottles, as they may compromise the integrity of the serum container.
4. Monitor the temperature in the water bottle using the thermometer to ensure a steady temperature.
5. During incubation, swirl the contents of both bottles every five minutes to ensure that the contents are evenly heated.
6. Once the bottle of serum has been at 56°C for 30 minutes, transfer the bottle of serum to ice to cool and proceed as needed. To prevent multiple freeze-thaw cycles, aliquot serum into smaller sterile containers, label with date and lot number, and transfer to a freezer (-20°C).

To prevent protein denaturation, which leads to turbidity and the presence of globules in the serum:

- Do not increase the speed of thawing using temperatures greater than 25°C in air, or 37°C in a water bath.
- Do not heat inactivate serum that is partially mixed/partially thawed.
- Do not heat inactivate serum at a temperature greater than 56.3°C.
- Do not inactivate serum for greater than 30 minutes.
- Swirl the serum during the heat inactivation process.