

# Heat Inactivation of Serum

## Protocol

CORNING

### Introduction

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 serum (FBS), bovine calf serum, rabbit serum, sheep serum and donor horse serum. There are also several different forms of serum subtypes defined by the types of processing available. FBS 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 metal ions, endotoxin, and proteolytic activity. Since serum is a blood product, it contains complement which can lead to complement-mediated cell lysis. To reduce this risk, serum can be heat inactivated. Heat inactivation destroys the complement, but the process 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

- Step 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, but it must be removed from the water bath as soon as it is thawed.
- Step 2: To start the heat inactivation process, prepare the water bath. Adjust the temperature of the water 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 sitting in the bath where the bottle of serum will sit.
- Step 3: Once the serum has thawed completely, mix it by gently swirling the container to ensure 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 it may compromise the integrity of the serum container.
- Step 4: Monitor the temperature in the water bottle using the thermometer to ensure a steady temperature.
- Step 5: During incubation, swirl the contents of both bottles every five minutes to ensure the contents are evenly heated.
- Step 6: After 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 -20°C freezer.

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 and/or partially thawed.
- Do not heat inactivate serum to a temperature greater than the specified temperature range of 56°C ± 2°C.
- Do not inactivate serum for greater than 30 minutes.
- Swirl the serum during the heat inactivation process.

Ordering Information

Products may not be available in all markets.

Cat. No.	Description	Storage	Size	Qty/Pk
35-010-CV	FBS (Fetal Bovine Serum) regular, USDA Safety Tested	-40°C to -10°C	500 mL	1
35-015-CV	FBS, premium, US Origin	-40°C to -10°C	500 mL	1
35-070-CV	FBS, premium, US Origin (gamma irradiated)	-40°C to -10°C	500 mL	1
35-071-CV	FBS, premium, US Origin (dialyzed)	-40°C to -10°C	500 mL	1
35-072-CV	FBS, premium, US Origin (charcoal stripped)	-40°C to -10°C	500 mL	1
35-073-CV	FBS, premium, US Origin (ultra low IgG)	-40°C to -10°C	500 mL	1
35-075-CV	FBS, premium, US Origin (tetracycline negative)	-40°C to -10°C	500 mL	1
35-076-CV	FBS, premium, Australia Origin	-40°C to -10°C	500 mL	1
35-096-CV	FBS, premium, Australia Origin (gamma irradiated)	-40°C to -10°C	500 mL	1
35-078-CV	FBS, premium, New Zealand Origin	-40°C to -10°C	500 mL	1
35-098-CV	FBS, premium, New Zealand Origin (gamma irradiated)	-40°C to -10°C	500 mL	1
35-030-CV	Donor Horse serum, US Origin	-40°C to -10°C	500 mL	1
35-046-CV	Rabbit serum, US Origin	-40°C to -10°C	500 mL	1
35-042-CM	Sheep serum, US Origin	-40°C to -10°C	1L	1
35-053-CM*	Bovine Calf serum, iron-fortified, US Origin	-40°C to -10°C	1 L	1
35-055-CM*	Bovine Calf serum, iron-fortified, US Origin (gamma irradiated)	-40°C to -10°C	1 L	1

\*Please inquire for lot-specific expiration dates or view the current certificate of analysis at [www.corning.com/lifesciences/media](http://www.corning.com/lifesciences/media).

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