

Methods for Storage, Thawing, and Freezing of Serum Products

Fetal Bovine Serum (FBS) and other serum products should be stored and handled correctly to assure long-term stability and to preserve growth performance characteristics throughout its shelf-life. This protocol briefly outlines crucial tips to maintain fetal bovine serum quality during the storage, thawing, and freezing of serum products from R&D Systems. These tips for handling fetal bovine serum also apply to all other serum products from R&D Systems:

[View All Fetal Bovine Serum Products](#)

[View All Serum Products](#)

Storage of Serum

1. Fetal bovine serum is most stable when stored frozen and protected from light until needed. The recommended storage temperature for serum is between -5 °C and -20 °C.

Note: Storage of serum below -20 °C may result in breakage of the bottle due to increased brittleness of the plastic bottles at these temperatures.

2. Repeated freeze-thaw cycles should be avoided as they will cause a decrease in serum performance and an increase in insoluble precipitates.
3. Fetal bovine serum should never be stored in a frost-free freezer, since this type of freezer repeatedly goes through defrost cycles that expose serum to freezing and thawing.

Thawing Serum

1. Remove the serum from frozen storage and thaw in a water bath with temperatures ranging from room temperature to 37 °C.

Note: Frozen serum should be thawed rapidly to avoid prolonged exposure of serum nutrients to higher salt concentrations during the thaw period.

2. Periodically agitate the bottle during the thawing process to re-suspend the viscous solutes and to avoid the formation of salt, protein and lipid gradients that can lead to excessive precipitation.
3. Promptly remove the serum from the water bath as soon as the serum is completely thawed.
4. Thoroughly mix the thawed serum before it is added to a culture medium or heat inactivated [[View Heat Inactivation of Serum Products Protocol](#)] as serum components such as proteins and salts tend to concentrate at the bottom of the container during thawing.

Note: Thawing of fetal bovine serum at temperatures above 37 °C is not recommended. This process may degrade heat labile nutrients, thus compromising the integrity and performance of the product, and can cause increased precipitate formation.

5. Once serum is thawed, it should be used promptly or divided into single-use aliquots and refrozen.

Note: Unused portions of thawed fetal bovine serum may be stored at refrigerated temperatures (2 to 8 °C) for a short period of time. However, the length of time that serum can be stored refrigerated with acceptable cell culture results varies with different cell lines.

6. Avoid thaw-freeze cycles or long periods of refrigeration as this can result in a decrease of stability and performance of the serum.

Freezing Serum

Fetal bovine serum and other serum products should be frozen as rapidly as possible to avoid prolonged exposure of serum nutrients to higher salt concentrations. Water is the first serum component to freeze will become less dense, resulting in other serum components such as proteins and salts to accumulate at the bottom of the container at a higher concentration. Therefore, slow freezing will result in excessive formation of crystalline precipitates.

Protocol Note: Precipitates & Flocculent Material in Serum Products

Fetal bovine 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.