

# Technical Bulletin

## Storing and Thawing Serum

Animal serum is an excellent source of nutrients for cells in culture because it contains proteins, lipids, salts, vitamins, minerals, amino acids and other components necessary for growth. When stored and handled correctly, the performance characteristics of serum can be maintained for many years. Improperly storing and/or thawing serum products can decrease not only the immediate and long-term stability, but also their effectiveness.

Growth promotion data demonstrate that serum maintains its growth characteristics throughout its shelf life if stored correctly.

The table below shows stability data for Fetal Bovine Serum (FBS) for a five-year period. To assure stability, sera must be correctly stored and handled.

### Storage

To effectively preserve the integrity of animal serum, it should be stored frozen and protected from light. The recommended storage temperature is -10 to -40 C. At temperatures below -40 C, the bottles may become brittle resulting in an increased risk of breakage.

Multiple thaw/freeze cycles should be avoided as they will hasten the degradation of serum nutrients and can induce the formation of insoluble precipitates. For this reason, serum should never be stored in “frost-free” freezers. These appliances occasionally warm themselves to avoid internal ice deposits and are detrimental to the clarity and stability of frozen serum products.

### Recommended Thawing Procedure

1. Remove the serum bottles from the freezer and allow them to acclimate to room temperature for approximately 10 minutes.
2. Place each container in a 30 to 37 C water bath or incubator. Excessive temperatures will degrade heat labile nutrients. If using a water bath, prevent the bottle caps from being completely submerged.
3. Gently swirl or shake the bottles every 10 - 15 minutes until the serum is completely thawed.

LONG-TERM STORAGE OF SERA							
DESCRIPTION	TEST	TIME 0	TIME 1 YEAR	TIME 2 YEAR	TIME 3 YEAR	TIME 4 YEAR	TIME 5 YEAR
Irradiated FBS	Multiple Passage	103	89	96	102	100	100
	Plating Efficiency	94	92	98	100	65*	41*
	Cloning Efficiency	48	113	105	95	28*	22*
FBS	Multiple Passage	96	145	97	93	104	122
	Plating Efficiency	100	98	35*	37*	33*	44*
	Cloning Efficiency	89	92	25*	43*	23*	24*

\*Growth data is % of control except \* which is % Plating Efficiency and Cloning Efficiency.

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## Cryoprecipitates

The method used to thaw serum is crucial to its optimum performance. The key to proper thawing is periodic agitation. If a bottle of serum is not periodically shaken or swirled as it thaws, salt and protein gradients will form throughout the liquid portion. Within these gradients are high concentrations of salts, proteins and lipids which can lead to the formation of crystalline or flocculent precipitates. These "cryoprecipitates" are not toxic to cell cultures, but they affect the appearance and consistency of each bottle of serum.

Small amounts of cryoprecipitates are not uncommon, even in serum that is thawed using the recommended procedure. This is normal and will not affect product performance.

If serum is thawed incorrectly, a much greater amount of cryoprecipitate will form and is often insoluble. Filtering serum to remove cryoprecipitates is not recommended and could result in the loss of nutrients, such as growth factors, mitogens and other proteins.

## Additional Recommendation

After thawing, use the serum promptly. Liquid serum may be stored refrigerated (2 to 8 C) up to four weeks. To avoid thaw/freeze cycles or long periods of refrigeration, it is recommended that any unused serum be immediately dispensed into small, useful aliquots and refrozen for future use.

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