GEMINI BIO-PRODUCTS

Precipitates in Serum

Introduction

The most important question is, "will precipitates in serum cause harm to my cells?" The answer to this question is no. Serum is an excellent supplement for cell culture media because it contains many of the components necessary for cell growth (proteins, electrolytes, lipids, minerals, vitamins, hormones, and many undefined growth factors). It is important to note specifically what these components are, as each of these has different physiological properties, affecting the appearance of serum and ultimately how Gemini processes and stores serum. In order to give a full understanding of why you might see precipitates and/or turbidity in serum, it is worthwhile to look at how companies process serum; what specifically causes precipitate to form; and how can we reduce the amount generated during the storage and use of serum.

How does Gemini process its serum?

The goal of collecting and processing serum is to produce a very consistent product that is visually clear and retains all of its cell growth promoting properties. For the sake of quality and consistency, Gemini's processing protocols and written standard operating procedures (SOPs) comply with Good Manufacturing Practices (cGMP).

Here is a brief overview of how our serum is processed:

1. Serum is collected as whole blood by direct cardiac puncture;
2. Collected material is cooled on ice and allowed to clot;
3. The supernatant raw serum is separated from the clot by centrifugation at 4C
4. Harvested serum is sterile filtered through a series of progressively finer membranes, culminating in the passage through three successive 0.1 micron filtration cartridges;
5. Sterile-filtered serum is aseptically dispensed into cell culture grade PETG bottles and frozen to a temperature of -20C.

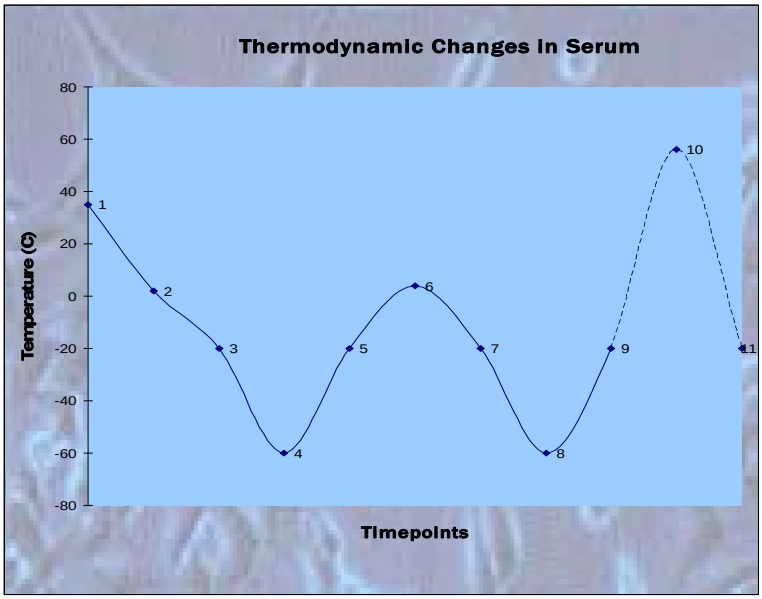
It is the rapid processing of the serum under cold, controlled conditions that is partially responsible for precipitate development. More about this in a minute…

We are confident that our processing and collection methods deliver one of the highest quality products available in the market.

What causes precipitates?

Precipitates can be found in all serum, apart from who the supplier is. A variety of different factors can cause precipitates and/or turbidity to develop in serum. Following are some of the most common factors contributing to this phenomenon.

A very common source of serum turbidity is cryoprecipitate. This is comprised of high-molecular weight plasma proteins that have solidified out of solution due to the low processing and storage temperatures common to serum manufacturing; remember that the serum is kept cold throughout all collection and manufacturing steps. One significant component of this precipitate derives from serum fibrinogen. Fibrinogen is soluble and often does not completely clot during early processing. When filtration occurs, the fibrinogen passes readily through even the finest of membranes. It is often not until after sterile filtration and subsequent freeze/thaw cycles have taken place that we see the fibrinogen aggregate and precipitate in thawed serum. At this point, the serum has typically been already bottled. This material appears in serum as larger flocculent masses, typically 1-2mm across. However, rest assured that there is no evidence that visible precipitate in serum has any affect on the performance of cells in the culture environment.



Processing Pathway	
1 to 2	Collection to Centrifugation
2 to 3	Decanting to storage
3 to 4	Shipment on DI to filtration facility
4 to 5	DI to Freezer at filtration facility
5 to 6	Pooling and filtration
6 to 7	Bottling and Stocking
7 to 8	Shipment on DI to research facility
8 to 9	DI to Freezer at research facility
9 to 11	Heat Inactivation (optional)

Another source of turbidity is prolonged incubation at 37°C. Calcium phosphate will often precipitate and can be seen as small opaque dots when viewed under a microscope. Again, there is no evidence that this precipitate has any impact on cultured cells.

Heat inactivation has been shown to cause turbidity in serum, as more heat-sensitive protein becomes denatured and leaves solution. Ask yourself if it is truly necessary to heat inactivate this material for your intended purpose?
Lastly, long term storage at 2-8°C increases the likelihood that various proteins and lipoproteins will denature and precipitate out of solution.
To summarize the above points, turbidity of serum is observed to increase in direct proportion to the number of times it undergoes significant thermodynamic change (i.e., freeze/thaw cycles). The graph above shows a typical freeze/thaw cycling pattern seen in serum.

How does one reduce the amount of precipitate generated during the storage and use of serum?

While these precipitates have not been shown to affect performance, it does affect the aesthetics of the serum and photomicrographic documentation. The following recommendations are intended to minimize precipitation and turbidity:

1. Avoid repeated temperature changes (i.e., freeze/thaw cycles)

2. Properly store and thaw the material. The bottles should be stored at -20 °C to insure the integrity of the serum is maintained. To thaw the material, remove the bottles from freezer and allow them to adjust to room temp.; put each bottle in an incubator/water bath at 30-37°C; gently invert/swirl the bottles every 15 minutes until the serum is completely thawed.

3. Re-think heat inactivation. The protocol of heat-inactivation originated before the advancements in serum processing implemented today. This makes the majority of reasons for heat inactivation no longer necessary.

With over 20 years of cell culture experience, we are confident that every step of our processing, from collection to the customers' receipt, delivers the highest quality product. The issue of precipitates in serum is very common to suppliers of serum and will continue to be for the foreseeable future. It is important to understand what causes this turbidity and what steps can be taken to reduce it.