![Avantor logo]

Avantor Seradigm Product Information

Bovine serum: Product and intended use

Fetal Bovine Serum (FBS) is a commonly used growth supplement in cell culture. FBS is derived from the blood of source animals. Avantor Seradigm serum products are intended for use in upstream research and biopharmaceutical manufacturing applications. Not for human consumption.

Storage and handling

Store at -10 to -20°C. Serum products should not be stored in a frost-free freezer because temperature cycling could affect the quality of the product. All serum should be used by the expiration date on the label and multiple freeze/thaw cycles should be avoided to avoid degradation of the product and the creation of insoluble precipitates. It is recommended that serum be used immediately after opening, however it can be stored at 2-8°C and should be used within 6-8 weeks after opening. Although there are no known hazardous ingredients in animal serum, common sense should be used during handling. All Avantor Seradigm serum products are sterile filtered. However, sterility is not guaranteed after opening, therefore, the use of aseptic techniques while handling is recommended.

Thawing procedure

Serum can be thawed immediately after removing from the freezer or it can be thawed slowly by removal from the freezer to overnight storage in a refrigerator (2-8°C). If thawing immediately, remove product from the refrigerator and allow it to sit out at room temperature for 10-15 minutes to become acclimated to room temperature. To completely thaw serum, transfer to a water bath that is between 30-37°C. Excessive temperatures will negatively affect heat labile nutrients. Swirl or gently agitate the serum every few minutes to completely mix serum constituents. If not properly mixed, turbidity and flocculent material may appear and proteins may settle at the bottom of the container. These do not affect the serum's performance in cell culture but will affect the appearance of the serum. Do not leave the serum in the water bath for any period longer than necessary to thaw the product. If smaller aliquots are needed, divide the product into sterile containers and refreeze immediately. If you choose to thaw serum at room temperature, it should be mixed regularly during the entire thawing period.

Heat inactivation

Heat inactivation is no longer necessary for most applications. Its intended purpose was to inactivate complement but improvements to serum manufacturing processes have eliminated the need to heat inactivate. An excellent tutorial, "Heat Inactivation of Serum: Are You Wasting Your Time?" addresses these concerns and helps clarify whether this process is still necessary for your application. The article can be found at **www.ResearchGate.net.** If heat inactivation is deemed necessary; we recommend following this protocol (which can be found at the address cited above):

– Thaw serum and mix the contents of the bottle thoroughly.
– While the serum is thawing, prepare a control bottle containing water. The control bottle should be stored along with the serum bottle to ensure identical beginning temperatures. This control bottle will be used to monitor the temperature and should be identical to the serum bottle, i.e. size, bottle material.
– Place the bottle of serum and the control bottle in a 56°C water bath containing sufficient water to immerse the bottle above the serum level. Suspend a thermometer or thermocouple in the water bottle. The thermometer should not touch the sides or bottom of the bottle.
– Swirl the bottles every 10 minutes for FBS and bovine calf serum and every five minutes for equine serum to ensure uniform heating of the serum.
– Monitor the temperature of the control bottle closely and begin timing when the temperature reaches 56°C.
– After 30 minutes at 56°C, immediately cool the bottle of serum in an ice bath. Do not heat at higher temperatures or for a longer period as serum may gel.

Precipitates in serum

Fibrin
Fibrin appears in serum as larger material (up to 1-2 mm) that is visible to the naked eye. Because serum is collected rapidly and processed under cold conditions, some fibrinogen (the soluble precursor of the clot-forming protein, fibrin) can remain in solution during the production process and then complete the clotting process after the final filtration, thus ending up in your bottle of serum as fibrin.

Calcium Phosphate
Calcium phosphate is also a common precipitate. It will appear as a general cloudiness in the serum that will increase upon incubation of the serum at 37°C. This precipitate will appear as small black dots when observed with an inverted microscope. These dots can appear to be moving due to Brownian motion. This type of precipitate is often mistaken for microbial contamination.

Other
Other precipitates can occur in serum such as fatty ester of cholesterol and some protein precipitates.[1]

Flocculence
Flocculence (or cloud-like masses) may appear in serum for several reasons. The most common of these is due to the denaturation of serum lipoproteins. Flocculence does not affect cell culture and can be removed by briefly centrifuging the serum at 400g then filtering the resulting supernatant along with your media. Do not filter the serum alone as it may clog filters.[2]

1. "Precipitates in Serum Products". Application note S0802, ThermoFisher Scientific, 2007
2. "Fetal Bovine Serum FAQs". Gibco, Invitrogen Corporation, 2001

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