

## Heat Inactivated Fetal Bovine Serum

Cat. No. : 164223

Size : 500mL / 100mL

### General Information

<b>Product Form</b>	Liquid
<b>Serum Level</b>	Premium Plus
<b>Shipping</b>	Low temperature, sealed, protect from light
<b>Storage</b>	Store at $\leq -15^{\circ}\text{C}$ , storage for up to 5 years Store at $2-8^{\circ}\text{C}$ for no more than one month

### Product Characteristics

1. Low endotoxin, free from Bacteria, Mycoplasma, Bacteriophages, Viruses, and other Contaminants.
2. No added factors, hormones, antibiotics or other supplementary components.
3. It has been treated by the thermal inactivation process. By inactivating the complement system and certain enzymes within it, the interference to cell culture can be reduced.
4. After inactivation, some immune-active components in the serum have been destroyed, reducing the immune stimulation to cells. It is more suitable for the cultivation of sensitive cell lines, reducing cell damage and death.

### Product Acceptance

1. The serum's internal and external packaging should be intact, without any damage, cracks, leakage or seepage.
2. Upon arrival, the serum should be in a frozen or ice-water mixed state, and should not be fully thawed.
3. In the event of above issues, please take photos for documentation and contact us promptly for a timely replacement.

### Thawing Method

1. Please thaw the serum at  $2-8^{\circ}\text{C}$  environment. Thawing at higher temperatures is not recommended, as it may cause the serum to become turbid, increase precipitation, and degrade its quality.
2. Please shake the serum gently from time to time during the thawing process to ensure that the serum composition and temperature are uniform, thus reducing the production of precipitation. Be careful not to create bubbles when shaking.
3. Once thawed, serum should be used as soon as possible. Repeated freezing and thawing should be avoided to maintain its quality.
4. Serum should not be placed at room temperature for extended periods. After use, promptly return it to a  $2-8^{\circ}\text{C}$  environment.
5. High-temperature thawing, vigorous shaking, repeated freezing and thawing, and prolonged storage can all lead to a decrease in serum quality.

### Notes

1. This product is only used for scientific research or further research, not for diagnosis and treatment.

- To maintain the optimal performance of this product, please avoid repeated freeze-thaw cycles.

## Common Issues and Solutions

### 1. Optimal Serum Thawing to Preserve Product Integrity

- After removing the serum from the low-temperature freezer, initially place it in a 2-8°C refrigerator for 12-24 hours to partially thaw, then allow it to fully thaw at room temperature. During the thawing process, it is crucial to gently and periodically shake the serum to ensure uniform distribution.
- Never place serum directly from a -20°C freezer directly into a water bath, whether at room temperature or at 37°C. The rapid thawing in the water bath, with a temperature differential of 57°C (-20°C→ 37°C), can easily cause the serum to precipitate and compromise its quality.
- Placing serum directly from a low-temperature freezer into a 56°C water bath is an extremely detrimental practice, showing a lack of responsibility towards the serum and a blatant disregard for scientific protocols!
- Serum should be stored at temperatures below -15°C. If the entire bottle cannot be used at once, it should be aliquoted under sterile conditions and stored frozen to avoid repeated freeze-thaw cycles.

### 2. What precipitates occur in serum?

- Fibrin is a typical larger precipitate that can reach 1-2 mm in size, and is visible to the naked eye.
- Calcium phosphate, another common precipitate, typically causes the serum to appear cloudy and may increase when cultured at 37°C. Under an inverted microscope, these precipitates appear as small black dots that, due to Brownian motion, may appear to move, often leading to a misidentification as microbial contamination.
- Cholesterol, fatty acid esters, and certain proteins are also common causes of precipitates in serum. Regarding cell growth, our experiments and experience indicate that these precipitates do not affect cell culture. This has also been confirmed by our customers and serum manufacturers.

### 3. What to do if flocculent precipitates appear after thawing serum?

- If you wish to remove these flocculent precipitates, aliquot the serum into sterile centrifuge tubes and centrifuge at 400-600 g for 5 min. The supernatant can then be added to the culture medium for cultivation.
- It is not recommended to remove these flocculent precipitates through filtration as it may clog the filter membrane. Additionally, filtration could lead to the loss of certain nutritional components in the serum.

### 4. Why heat-inactivate Serum? Is heat inactivation necessary?

- Heating inactivates the complement system in serum, rendering it inactive. Typically, active complement components can stimulate smooth muscle contraction, release histamine from mast cells and platelets, activate lymphocytes and macrophages, and contribute to the process of cell lysis.
- Numerous studies have shown that most cell cultures do not require serum heat inactivation. However, heat-inactivated serum is recommended in immunological research for the cultivation of embryonic stem (ES) cells, insect cells and smooth muscle cells. Experiments have demonstrated that serum treated with proper heat inactivation has only a minimal or no promoting effect on cell growth. However, high temperature treatment usually affects the quality of serum, resulting in reduced in cell growth rate. Furthermore, heat-treated serum often shows a significant

increase in precipitates, which appear as "small dark spots" under an inverted microscope, which often makes researchers mistakenly think that the serum has been contaminated. When the serum is placed at 37°C, the precipitates will increase further potentially leading to the misconception that microbial division and proliferation are occurring. Therefore, we recommend avoiding heat treatment unless absolutely necessary, as it saves time and ensures the quality of the serum.

**5. How to avoid the formation of precipitate in serum?**

Excessive temperature, prolonged incubation and uneven shaking can all lead to an increase in precipitation. We recommend that serum heat inactivation should be avoided unless absolutely necessary. If heat inactivate is required, it should be strictly performed at 56°C for 30 minutes, with gentle and continuous shaking to ensure uniformity.

**6. What is the best way to store serum?**

Serum intended for long-term storage must be stored at -15°C or lower. Studies have shown that serum stored at -80°C does not exhibit any change in performance, but the large temperature differential during thawing can lead to increased precipitate formation. Therefore, it is not recommended to store at -80°C. When stored at 2-8°C, serum should not be kept for more than one month. If the entire bottle cannot be used at once, it is advised to aliquot and store it to avoid repeated freeze-thaw cycles. Additionally, serum volume increases by approximately 10% upon freezing, so leave sufficient space when aliquoting.

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