

Purpose

The following protocol is suggested for the heat inactivation of bovine serum. Bovine serum is heated to $56 \pm 2^\circ\text{C}$ for 30 ± 2 minutes in a water-bath.

Note: Our heat inactivation procedure may not be the same as the one your lab is using. Keep in mind that heat inactivation requires the serum to be at 56°C for 30 minutes. Prior to reaching 56°C , the serum is in a water bath at elevated temperatures for approximately 1 hour, the time it takes for the serum in the bottle to reach 56°C . This is very different than putting the serum in a 56°C water bath for 30 minutes, which is not heat inactivation by definition. We are not responsible for inconsistencies in performance when compared with serum using a different procedure.

Definition

Heat Inactivation (HI) – a process by which serum is maintained at a temperature of $56 \pm 2^\circ$ for 30 ± 2 minutes.

Equipment

- Water bath capable of maintaining temperatures of $56 \pm 2^\circ\text{C}$. Preferably a shaking water bath.
- Calibrated thermometers capable of measuring 56°C .

Materials

- Control bottles should be equivalent to the product bottle. Control bottles are volumed to the same level as the product being heat inactivated and fitted with a thermometer suitable for monitoring 56°C . The thermometer should not touch the sides or bottom of the bottle.
- Circular lead weights

Specifications

The temperature of the control must be $56 \pm 2^\circ\text{C}$ for 30 ± 2 minutes during the heat inactivation process.

Precautions

For laboratory use only

Procedure

1. Thaw serum. *If serum was thawed in a refrigerator allow serum to come to room temperature prior to placing in water bath.*
2. Fill the water-bath with sufficient water so that the product and control bottles are immersed near the serum level.
3. Set water-bath temperature to maintain the product at $56 \pm 2^\circ\text{C}$.
4. Place a lead weight on the control bottle and place into the center of the water bath.
5. Mix the contents of the product bottles using a gentle swirling motion until the product is uniform.
6. Place lead circular weights over the tops of the bottles to keep them upright.
7. Place the bottles in the water-bath.
8. Swirl bottle thoroughly every 10 minutes or if applicable turn on the oscillating shaker unit. Check the temperature of the control bottle frequently as the temperature approaches $56 \pm 2^\circ\text{C}$.
9. When the temperature of the control reaches $56 \pm 2^\circ\text{C}$ start the timer for 30 minutes. If a shaking water bath is not available, ensure bottles are swirled every 10 minutes during the entire process.
10. After 30 minutes turn off the oscillating shaker and remove the bottles from the water bath. Cool to room temperature and either aliquot or return to the freezer.

Denatured protein resembles a jelly like substance, usually at the bottom of the bottle of serum. If this occurs, decant serum and use. Denatured protein should not be confused with Fibrin, a fine precipitate sometimes seen in thawed serum. Fibrin precipitate is a fine whitish powder flake, which has no ill effects on cell culture.