

Heat Inactivation Protocol

Materials Required

- Large temperature-controlled water bath. The addition of the frozen serum can cause a decrease in water temperature, so be sure to use a large enough water bath where the addition of serum will not cause a drastic change.
- Control bottle with the identical volume (100 mL) as the serum bottle(s) intended to be heat inactivated.
- Thermometer

Recommended Protocol

When preparing GMP Human AB Serum for use, gradually thaw, either at room temperature for several hours or in 2 – 8 °C conditions overnight. Gently mix product by swirling (to minimize foaming) after thawing.

1. Thoroughly thaw serum and gently swirl to homogenize. Take care to avoid bubbles and/or foaming.
2. Prepare the control bottle containing water. The control bottle should be stored along with the serum bottles to assure identical initial temperatures. This control bottle will be used to monitor the temperature and should be identical to the serum bottle(s) (e.g., PETG bottle of the same volume with a cap or stopper).
3. Place the bottle(s) of serum and the control bottle into a 56 ± 2 °C water bath containing sufficient water to immerse the bottle above the serum level but still below the level of the cap/threading to ensure sterility. Suspend a thermometer in the water bottle. The thermometer should not touch the sides or bottom of the bottle.
4. Swirl the bottles gently every 3-5 minutes to ensure uniform heating of the serum.
 - a. Caution: Failure to keep serum adequately mixed during this step could cause an increase in precipitates.
5. Monitor the temperature of the control bottle closely and begin timing when the temperature reaches 56 ± 2 °C.
 - a. Caution: Minimize the time it takes for the overall water bath to reach the temperature back to 56 ± 2 °C
6. After 25-30 minutes, remove the serum bottle(s) and slowly cool to room temperature.
 - a. Caution: Exceeding this amount of time could cause reduced serum performance.
7. Afterward, place serum at 2 – 8 °C. The serum should be left to equilibrate overnight before aliquoting. If the serum is to be filtered to remove precipitates, first it must be cooled to 4 °C overnight, then filtered and aliquoted. However, we do not recommend filtering the serum. Seeing precipitates after heat inactivation is normal. Heat inactivated serum may be re-frozen once, thawed and then used.
8. Heat inactivated serum should be stored at ≤ -10 °C; otherwise, the amount of accumulated precipitate will increase as the serum stands in the thawed state, even if left at 2 – 8 °C. We do not recommend more than one freeze-thaw cycle after heat inactivation.