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## Heat Inactivate FBS

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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

Heat inactivate FBS to denature compliment. Critical for trypanosomes but not for EA.hy 926 cells.

## Troubleshooting

## Before start

Thaw FBS in the fridge.



- 1 Allow serum to acclimate at room temperature for a minimum of 10 minutes or refrigerate overnight at 2° C to 8° C.
- 2 The serum may then be completely thawed at room temperature. Or, you may choose to proceed by thawing the serum in a water bath at 37° C. Thawing serum at temperatures above 37° C or for an extended period of time could lessen the quality of serum and possibly cause increased amounts of precipitates and/or cause a cloudy appearance to the serum. Swirl the bottles throughout the thawing process every 10-15 minutes as this will disperse the released salts and proteins uniformly.
- 3 Prepare a water bath, if not already done so during the thawing process, that can be temperature controlled to 56° C for the heat inactivation process. There must be enough water to submerge the entire contents of all bottles.
- 4 Fill a control bottle with water to monitor the water bath temperature. The control bottle must be identical to the bottles being heat inactivated and the temperature of the water within the control bottle should be the same as that of the thawed serum.
- 5 Place the control bottle and serum into the 56° C water bath. Suspend a calibrated thermometer into the control bottle to monitor the temperature during the heating process. Do not allow the thermometer to touch the sides or bottom of the bottle.
- 6 Set a timer to 30 minutes once the temperature of the control bottle has reached 56° C. Maintain this same temperature the entire duration of the process.  
Gently swirl the bottles every 3-5 minutes to make certain the serum remains uniform throughout heating.
- 7 Remove the heat inactivated serum after 30 minutes and gently swirl once again.
- 8 In cell culture hood, aliquot 50 mls into 10 50 ml containers.
- 9 Label as Heat Inactivated FBS
- 10 Store the heat inactivated serum at -20° C in frosted freezer. Caution to Note: The following possibilities may cause protein precipitates:
- 11 Caution to Note: The following possibilities may cause protein precipitates:
  - 1.) Improper Thawing
  - 2.) Inactivating serum that has not been thoroughly mixed homogeneously
  - 3.) Inactivating serum above the recommended temperature

30m



4.) Inactivating serum for a longer period of time than recommended 30 minutes