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Heat Inactivate FBS



Forked from [Heat Inactivate FBS](#)

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Paolo Edoardo Ginatta Arosemena: Edited and simplified protocol for our laboratory needs

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External link: http://www.serumsourceintl.com/pdf/heat_inactivation_instructions.pdf

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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.





Thaw

- 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  37 °C in a water bath. 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 in for the heat inactivation process. There must be enough water to submerge the entire contents of all bottles.

Heat-inactivate

30m

- 4 Place the serum into the  56 °C in water bath for  00:30:00
- 5 Gently swirl the bottles every 3-5 minutes to make certain the serum remains uniform throughout heating.
- 6 Remove the heat inactivated serum after the 30 minutes and gently swirl once again.
- 7 In cell culture hood, aliquot 50 mls into 10 50 ml containers.
- 8 Label as Heat Inactivated FBS

30m

Causes of Failure

- 9 Improper Thawing
- 10 Inactivating serum that has not been thoroughly mixed homogeneously



- 11 Inactivating serum above the recommended temperature
- 12 Inactivating serum for a longer period of time than recommended 30 minutes