



VERSION 2

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Protocol status: Working
We use this protocol and it's working

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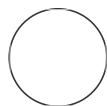
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Heat-inactivation of Fetal Bovine Serum (FBS) V.2

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ABSTRACT

The objective of heat inactivation is to destroy complement activity in the serum without affecting the growth-promoting characteristics of the product. Removal of complement activity from serum, such as fetal bovine serum, is not required for most cell cultures but may be necessary for cultures that are sensitive to the complement activity. Since heat inactivation of the serum may, to some extent, decrease the growth performance properties of the serum, this procedure should only be performed if required for optimal cell growth. Researchers should evaluate the applicability of heat inactivation regarding their own application.

GUIDELINES

Do not use heat inactivated FBS unless necessary.

MATERIALS

Circulating hot water bath

Timer

Thermometer

Ice bath

SAFETY WARNINGS




58 degrees Celsius is hot. Take care when working with these temperatures.

BEFORE START INSTRUCTIONS

Clean and sanitize everything

FBS heat-inactivation procedure

2d 3h 24m

1 Slow thaw FBS at  4 °C overnight

1d 0h 42m

Note

It isn't necessarily necessary to slow thaw, but FBS can form precipitation with quick thawing. This doesn't impact the quality of the FBS, but personally I would slow thaw if possible.

2 Aliquot FBS into  50 mL units in a sterile environment



Note

You can choose whatever volume of units to aliquot, but 50 mL is great because it gives exactly 10% FBS percentage in a 500 mL medium bottle.


3 Prepare a volumetrically equal blank container with distilled water





Note

A volumetrically equal blank container is necessary to control the temperature within the FBS container, and not only the external temperature of the water bath.

4 Let FBS equilibrate with the distilled water container for  00:30:00 at  Room temperature

30m


5 Clean the circulating water bath. Fill with an appropriate amount distilled water. Set temperature at  56 °C


6 Set FBS aliquots and blank into preheated water bath at  56 °C for  00:30:00 . Use a thermometer to track temperature in distilled water blank, and start timer when temperature reach  56 °C . Agitate aliquots every  00:05:00 to ensure homogenous heating

35m

7 Remove aliquots from heat bath, and place FBS  On ice or  4 °C for  00:30:00

30m



8 Store aliquots at  -20 °C until use