

Latest findings on heat inactivation of sera

Updated information* from Biochrom AG

To achieve ideal cell growth, serum, e.g. Fetal Bovine Serum (FBS), is added to the culture medium of cell cultures. Sera are natural products, which feature, next to growth-promoting substances, undesired components.

The process referred to as “heat inactivation”, i.e. the heating of the serum, mitigates or eliminates a broad range of generally disturbing effects of the serum. Heat inactivation is used, for instance, to inactivate the serum’s complement binding capacity.

However, heat inactivation has positive as well as negative effects on FBS. Biochrom AG does thus not recommend a general inactivation of FBS. An effective alternative is the irradiation of FBS.

1 Usage of FBS in cell culture

Fetal Bovine Serum (FBS) is the most commonly used supplement (2-20 % in medium) in cell culture systems. Where applicable, it is also used for the production of therapeutic proteins. FBS is a highly complex mixture of serum proteins, amino acids, peptides, growth factors, hormones etc. This is why FBS represents a supplement in cell culture that can be used universally.

Biochrom AG’s FBS is based on a thorough selection of the respective raw serum. Aseptic serum filling is performed within an area of cleanliness level A with a background environment B in accordance with the supplementary GMP guidelines for the manufacture of sterile pharmaceuticals, annex 1. All sera are tested for a potential contamination with mycoplasma. Sera are only released if the result is negative.

2 Heat inactivation

Sera are undefined natural products, which implies that they are not only composed of growth-promoting substances, but also of undesired components. Heat inactivation mitigates or eliminates disturbing effects of the serum.

Heat inactivation is the most common procedure used to inactivate the complement binding capacity of the serum in order to ensure that cells are not being lysed by means of antibody binding. Research has shown, however, that fetal calf serum contains only a few components of the complement system (1). Nevertheless, complement inactivation may be of significance if the experiment set-up includes, for example, a screening of specific viruses or virus propagation.

During the heat inactivation process, lactate dehydrogenase (LDH) is being destroyed. This is advantageous if the user intends to determine the LDH level in the cell culture supernatant (2).

However, heat inactivation also causes a loss of valuable components: vitamins are partly or fully damaged, while growth factors are reduced in their concentration. It may also impair the capability of cells to attach to the surface of cell culture flasks (3).

In order to kill present mycoplasma, heat inactivation is, however, not required, as all sera produced by Biochrom AG are free from mycoplasma as a result of commercial filtration procedures and compliance with top quality standards.

Considering all benefits and disadvantages, it becomes evident that the user should decide case by case whether or not to resort to heat inactivation. A general inactivation is not recommended.

In order to inactivate viruses, irradiation of serum offers an alternative to heat inactivation. The dose of 30 to 40 kGy specified for the irradiation, which is performed in a facility authorised by Biochrom AG, does not damage the serum detectably.

If the user resolves to use heat inactivation, Biochrom AG recommends the following procedure:

Perform heat inactivation at 56 °C for a period of 30 minutes by heating the laboratory-scale serum bottles in a water bath. During this process, stir the serum and shake it at least from time to time, while avoiding foaming. It is important that the serum (rather than the water bath) has the desired temperature during the entire period.

3 Literature

1. Triglia, R.P., Linscott, W.D.: *Titers of nine Complement Components, Conglutinin and C3b-Inactivator in adult and fetal bovine Sera*. Molecular Immunology. 17: 741-748 [1980] Elsevier. 1980.
2. Lindl, T., Gstraunthaler, G.: *Zell- und Gewebekultur*. 8. Auflage. Heidelberg: Spektrum Akademischer Verlag. 2008.
3. Giard, D.J.: *Routine Heat Inactivation of Serum reduces its Capacity to Promote Cell Attachment*. In vitro cellular & developmental biology : journal of the Tissue Culture Association. 23: 691-697 [1987]. Tissue Culture Association. 1987.