

Fetal Bovine Sera and Substitutes

Collection

Gemini Bio-Products uses animal sera which have been collected from the continental United States as well as sources outside the US approved by the United States Department of Agriculture (USDA). Collection facilities outside the United States are in countries that have been declared free of certain bovine viruses such as foot and mouth disease by the USDA. These sources are all within North America. Each lot of animal serum is certified as to country of origin, assuring when applicable, that USDA importation regulations are strictly followed. Documentation of traceability can be supplied upon request for every lot of Gemini fetal bovine serum.

Fetal bovine serum is collected using a closed, aseptic cardiocentesis method in order to minimize hemoglobin, endotoxin, and microbial agents.

Processing

Serum processing is performed using Good Manufacturing Practices (GMP) at refrigerated temperatures. Serum is separated from the clot by centrifugation. Sterile filtration is performed using multiple, 0.1- μ m membrane filters (unless otherwise specified). Filtered serum is normally dispensed into cell-culture-grade PETG bottles and rapidly frozen to a temperature of -10 °C to -20 °C. Serum can be supplied in glass bottles upon request.

Growth Promotion and Cytotoxicity

Our serum is evaluated for cell growth promotion and/or cytotoxicity. Cell lines that are free of mycoplasma and adventitious viral agents are cultured to the equivalent of 3-4 population doublings in a medium containing 10% test serum. Cells are examined microscopically for atypical morphology and evidence of cytotoxicity. It is impractical to test sera or other products on every cell type in use by our customers. If you have a specific cell type you would like tested, such as Embryonic Stem cells, we will gladly discuss a modified testing

program to meet your needs. Because of the many diverse cell types in use and the variability of cell-line matching programs, we provide samples for your own evaluation. This is the only true measure of how sera or other products will perform for your specific application.

Sterility

The sterility of serum products is evaluated in accordance with Federal Regulations CFR 9 113.26. Statistical samples are taken during the aseptic filling process and tested for the presence of bacteria and fungi. Samples are inoculated into Thioglycolate broth and Soybean Casein Digest broth and incubated for up to 21 days at 21 °C and 37 °C, respectively. At the end of this period, cultures are examined and must be determined to be free of any microbial contamination before the product is released for sale.

Virus Screening

The screening of bovine serum for viral contamination is accomplished by cultivation of bovine cells known to be free of both Mycoplasma and adventitious bovine viruses. Cells are grown to the equivalent of 3 to 4 population doublings, using a minimum serum concentration of 15%. The viruses screened are BVD, PI3, and IBR. No viral screening method can be guaranteed to detect all viruses that may be present in a particular lot of serum. While we strive to use the most accurate and sensitive methods, each serum user should understand that any particular negative virus test is not an absolute guarantee of the absence of that virus.

Mycoplasma Testing

Mycoplasma testing is performed on serum using the Barile large-volume broth inoculation method. Any serum lots containing detectable mycoplasma are rejected and not released for sale.

Biochemical Analysis

Chemistry values including total protein content, gamma globulin content, species identification, hemoglobin content, osmolality, and pH are determined on all finished lots of serum. Effective April 2003, we began testing all new lots of fetal bovine serum for the presence of tetracycline; test results are reported on the Certificate of Analysis that accompanies the product.

Hormone Testing

Effective June 1, 2004, all GemCell™ US Origin (Cat# 100-500), BenchMark™ (Cat# 100-106) fetal bovine serum, and Fetalplex™ (Cat# 100-602) animal serum complex will be assayed for the following hormones: estradiol, insulin, progesterone, testosterone, thyroxine (T4), and triiodothyronine (T3). Results will be reported on the Certificate of Analysis that accompanies the product.

Endotoxin Testing

The presence of endotoxin is determined by the Limulus Amebocyte Lysate (LAL) gel

clot method. Each run contains a certified control performance standard.

Heat Inactivation

Gemini will provide heat inactivation for most sera upon request, as a service to our customers.

The practice of heat inactivating serum was originally developed when only serum from adult cows was available for cell culture. Adult serum contains immune factors, particularly serum complement, which may inhibit or destroy cells under certain conditions. Heating the serum to 56°C for 30 minutes is intended to inactivate serum complement.

Fetal bovine serum seldom benefits from heat inactivation and, therefore, the process is discouraged in most cases. Heat inactivation can decrease growth promotion ability, and add a considerable amount of precipitate from protein denaturation. We suggest the customer do a simple test to compare native serum to heat-inactivated serum.