

Animal sera

Fetal Bovine Serum (FBS, FCS), and also other sera types such as Newborn Calf Serum (NBCS) are still an essential supplement widely used in mammalian cell culture. They provide a unique mixture of growth factors, trace elements etc that is required to grow cells *in vitro* at acceptable costs. The delicate nature of most components, however, require storage below -20 °C. Under these conditions, animal sera can be stored for quite a long time; according to data on file, and also following international standards, FBS can be stored for at least 5 years without infringement of quality, and

cell culture performance. Most protocols for the usage of FBS in bioprocessing today require the exposition to γ -irradiation at a minimum dose of 30 kGray in a validated procedure. To keep optimal conditions, thawing, and freezing of serum in general should be kept short. Bioprocessing, on the industrial scale, is using FBS/NBCS in substantial quantities. As global demand for these types of animal sera has dramatically gone up over recent years, its use in bioprocessing for the manufacturing of therapeutic proteins is regulated by the authorities.

FBS/NBCS from Biochrom AG for bioprocessing application is almost exclusively sourced in Australia, covered by a Certificate of Suitability (CoS) issued by the EU health authorities. Raw serum collected in Australia, is imported to Europe, and sterile filtered using multistep process filtration at Biochrom's production facilities in Berlin.

Homogenous manufacturing lots of 2000 litres are standard; customers are invited to test its performance in cell culture, after the lot has passed Biochrom's strict quality control. Quality control (QC) has two parts: sterility control according to the regulations set out by the Ph. Eur., and testing for bovine adventitious virus contamination. QC results are laid down in a "Certificate of Quality" that is available alongside the serum sample.

Wherever FBS is used as supplement in bioprocessing, a detailed documentation is required covering the whole chain from the individual collecting blood bags all the way to the sterile filtered and tested end-product.

During serum harvesting, and centrifugation of fetal blood, it may easily become contaminated by bacteria and/or fungi. Careful thawing protocols, sterile filtration, and strict sterile control of the end-product is thus one of the key responsibilities of commercial serum suppliers.

As a leading manufacturer of FBS and other animal sera, Biochrom AG has learned the value of supplying diverse types of sera of the highest quality for use in cell culture. Each product has its own application universe and performance profile for cell growth. While FBS remains the most extensively used and effective additive for mammalian cell culture, Newborn Calf, Iron Fortified Newborn Calf, but also other sera types such as Donor Horse Serum or Porcine Serum are all vital products for a wide range of applications.

FBS and other animal sera support the growth of a wide range of cell lines. Serum has direct nutritive properties

for cells and indirectly facilitates solubility and metabolic utility of other media components. Serum in cell culture also protects cells and valuable recombinant proteins from



Pre-filtration of sera

degradation by serine proteases. Serum has a buffering capacity which renders cells less sensitive to the stresses of variation in gas delivery, viscosity of the media, and shear forces in large bioreactors.

The quality of Biochrom AG sera is demonstrated in low endotoxin levels, minimal lot-to-lot variability, and complete documentation in the form of a Certificate of Analysis and Certificate of Origin for raw materials. Biochrom's cell line screening program minimizes the need for multiple lot testing. As with all Biochrom AG products, rigid quality control measures are exercised at every stage of production in selecting raw material, manufacturing, testing, and document control. With hundreds or thousands of litres produced



Filling

in our facility, you can be confident that Biochrom AG sera meet or exceed all critical requirements for use in biopharmaceutical production.

Biochrom offers:

- Reliability demonstrated 30 years of continuous production.
- Extensive testing of raw material to assure quality and reproducibility.
- Only raw material from regularly inspected sources in Australia, and New Zealand.
- Manufacturing of sera under GMP guidelines.
- Use of multiple 0.1 micron validated filters to assure sterility and removal of mycoplasma.
- Manufactured and tested to ensure low endotoxin and freedom from adventitious agents.
- Large lot sizes to maximize reproducibility and uniform performance.

Sourcing

Biochrom AG sources serum based on official information from countries that are free from Bovine Spongiform Encephalopathy (BSE); mainly Australia. All material is from government inspected abattoirs or facilities. Animals receive both ante and post mortem inspections. Donor animals

are maintained in controlled environments inspected by appropriate government agencies. Biochrom recognizes the importance of a clear audit trail to verify the origin of raw material.

Documentation

Biochrom AG requires documentation from raw material suppliers and audits to confirm origin and health inspections. These documents allow Biochrom to trace the animal's

origin prior to slaughter. Biochrom continues to monitor the health status of all countries supplying raw material.

Collection

Biochrom AG FBS is collected using cardiac puncture method in closed sealed bags; Newborn Calf Sera are from open abattoir collection. Donor Horse Serum is collected

by venipuncture, screened and found negative for Equine virus types.

Processing

All raw material blood for sera is chilled upon collection. The blood is clotted then separated by centrifugation and pooled. The pooled serum is bottled and labelled immediately, then

frozen at -20°C . Samples of the pooled sera are tested. Only serum meeting Biochrom's high standards is approved for manufacturing.

Manufacturing

Biochrom AG manufactures all serum products at their sterile filtration facility in Berlin. Constantly reviews and upgrades the standard operating procedures (SOPs) and performance testing to guarantee the highest possible industry standards.

of 50, 100, 500, or 1000 ml, under validated class 100 clean-room conditions.

Raw serum is thawed and pooled, followed by a proprietary filtration process using validated 0.1 micron filters. Finally, the product is dispensed aseptically into sterile PET bottles

After filling, the bottles are immediately labelled and frozen at -20°C in a quarantine area. Product is released for sale when our quality assurance department issues a Certificate of Analysis confirming that the batch meets Biochrom's final product specifications.

Post-manufacturing steps

Biochrom AG will, upon customer request, heat inactivate or gamma-irradiate sera. Heat inactivation is performed at +56 °C for 30 minutes. Gamma-irradiated sera is typically exposed to a cobalt 60 source at a specified minimum dose of 30 kGray. Please inquire for more details.

Quality control

Every lot of serum is tested and released with a Certificate of Analysis reporting actual test results. Specifications for Biochrom's serum products are shown in table 49. A sample serum Certificate of Analysis is explained on pages 18.



Tab. 49: Biochrom AG animal sera specification summary

Product assay	FBS	Newborn Calf Serum
Identity for species electrophoresis profile	tested and reported	tested and reported
Protein (mg/dl)	3.2 - 4.5	3.5 - 8.5
Hemoglobin (mg/dl)	< 25	< 20
Endotoxin (U/ml)	< 10	< 20
Gamma-globulin	tested and reported	na
pH value	6.80 - 8.20	7.00 - 8.35
Osmolality (mOsm/kg)	280 - 340	260 - 340
Biochemical profile	tested and reported	tested and reported
Bacteria and fungi	negative	negative
Adventitious viral agents	negative*	negative
Mycoplasma	negative**	negative

* negative as per EMEA test guideline

** 21 CFR 610.30 and PTC

All Biochrom AG test methods follow strict Standard Operating Procedures outlined below:

• Biochemical tests

Each lot of processed serum is analysed for over 30 blood serum measurements. The examination is shown on the

sample Certificate of Analysis on pages 19 – 21.

• Endotoxin testing

Cell wall residues of Gram negative bacteria are commonly named “endotoxins”, and are another threat in the serum manufacturing process. Sloppy collecting and processing of the raw serum may result in a higher endotoxin burden of the respective serum lot. Endotoxins are very hard to remove from the serum, and are even capable to pass the different filtration steps. Endotoxins

may result in poor cell growth, and may also being passed to the end-product, often a therapeutic protein. All serum products are tested at the finished product stage by the *Limulus amoebocyte* lysate (LAL) test method and point “chromogenic” procedure. Results are reported in EU per ml, where 12 EU are roughly equivalent to 1 ng of endotoxin.

• Virus screening

A particular level of safety is required by the authorities as concerns the possible presence of adventitious bovine viruses in the serum. At least 17 different types of viruses are known to being able to penetrate the bovine placenta, and may be found in the fetal blood. The most common are Foot-and Mouth-Disease virus, Bovine Rhinotracheitis (IBR), Bovine Parainfluenza (PI3) as well as Bovine Diarrhoea virus (BVD). With respective geographical origins, contamination with Blue-Tongue-Virus (BTV) is also an issue.

All lots of Australian FBS intended for usage in bioprocessing are screened for adventitious virus according to EMEA/CVMP/743/00, EMEA/BWP 1793/02 and EP 5.2.5 guideline.

The comprehensive documentation is available for every single FBS lot together with the Certificate of Quality confirming sterility and absence of mycoplasma.

Ancillary products

In biopharmaceutical production, an increasing degree of control of all components is mandated by regulatory and cost considerations. Biochrom AG has responded to the needs for high quality, consistent, and cost-effective supplements. This includes Australian Bovine Serum Albumins (BSA), cell attachment factors, enzymes for cell dissociation, and trypsin inhibitor in bulk quantities.

Whatever cell culture component is required for your project on the industrial scale, our technical service will work with you to supply the product you demand.

The following information will be helpful in expediting your request:

- chemical identity
- raw material requirements and specification
- purity and other final product specification
- amount requested
- packaging considerations

