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Serum characteristic

Serum is the fraction of blood, remaining after coagulation and centrifugation of the cellular parts. It provides an extreme complex mixture of plasma proteins, growth factors, hormones etc. that makes it the commonly used supplement in cell culture. Components may vary according to origin, nutritive conditions and processing. Because of these natural variations it is necessary to do batch testing.

The high quality of Biochrom AG sera is based on the careful selection of raw serum, an optimized filtration process and sterile bottling: The aseptic filling of sera results in an area of the purity class A with a background environment of the purity class B according to the complementary GMP guidelines for the manufacture of sterile drugs, annex 1.

Growth support level of sera in cell and tissue culture is determined through extensive biological

testing, including growth tests with a range of commonly used cell lines. All sera have to test negative for mycoplasma. The release of the sera is only effected after a negative result.

Fetal Bovine Serum (FBS) and Newborn Calf Serum are examined to exclude viruses such as Bovine Virus Diarrhoea (BVD-MD), Bovine Herpes Virus type 1 (BHV-1), and Parainfluenza Virus type 3 (PI-3). Test results have to prove negative with respect to cytopathogenicity and immunoperoxidase staining. FBS is screened also for antibodies against these virus types.

For FBS, an independent laboratory raises a range of physicochemical data, provided to customers together with the sample.

Details for sera

- **Sterile filtration**

Like all liquid products (media, additives), animal sera are sterilized using the membrane filtration technique with pore size $< 0.2 \mu\text{m}$ for sera in general, media and additives. For FBS, a filter pore size with $0.1 \mu\text{m}$ is used as standard.

- **Sterility control**

Aliquots of each lot are inoculated into tryptone soya broth and thioglycollate. The full sterility control procedure routinely takes 14 days. All products of animal source like serum or trypsin are screened through standard procedures for mycoplasma contamination.

- **Storage**

Due to their sensitivity against enzymatic decomposition, animal sera have to be stored at temperatures -20°C . All data prove excellent stability, and performance of the sera for at least 60 months.

- **Sampling and shipment**

Samples are available for all sera, FBS in particular, free of charge. To order test samples, please use the copy form on page 173, since it contains all specifications required. Your reservation will be kept for the testing period. Shipment of sera is made at $\leq -20^\circ\text{C}$ on dry ice.

- **Thawing and freezing of sera**

To prevent formation of high salt concentrations, the thawing procedure should be kept short. We recommend to warm up the bottle for about half an hour at room temperature (until a liquid film has formed), then put it into the water bath.

Tab. 1: Serum-thawing times for different filling quantities

Thawing	Thawing times		
	100 ml	500 ml	1000 ml
1. Thawing up to a formation of a liquid film			
at room temperature	35 min	90 min	120 min
in a water bath at +15 – +20 °C	5 min	18 min	35 min
2. Following time for warming up to +20 °C in the water bath	15 min	25 min	45 min
3. Following time for warming up to +37 °C in the water bath	20 min	35 min	40 min
The bottles should be immersed in the water bath up to the serum level in the bottle. During warming, the content should be mixed by rotating slowly (do not shake to prevent formation of foam).			

Re-freezing of sera:

Serum should be frozen quickly. A pre-cooling at +2 – +8 °C is recommended. Frequent freezing and thawing cycles have to be avoided: preferably aliquote after first thawing.

Tab. 2: Sera freezing duration (initial temperature approx. +20 °C)

Freezing	Volume		
	100 ml	500 ml	1000 ml
with pre-cooling to +2 – +8 °C	120 min	180 min	210 min
without pre-cooling	150 min	225 min	270 min

- **Heat inactivation**

Sera are undefined natural products. As such, in addition to containing growth-promoting substances they can also contain undesirable components (e.g. complement system).

In order to reduce the content of interfering substances, many experimental procedures stipulate inactivation of the serum prior to use, using the so-called “heat inactivation” process. The serum is normally heated to +56 °C for 30 minutes.

- **Request for samples to test new batches**

Please fill in the form to request samples (see appendix) and send it to us via fax.

Or request samples online: fill in the form www.biochrom.de/products/sera/sample-order-fbs.

Fetal Bovine Serum (FBS)

Fetal Bovine Serum (FBS) is the most applied supplement (2–20 % in medium) in cell culture systems and where applicable also in the production of therapeutic proteins. FBS is a byproduct of the meat industry and can be extracted as such in sufficient quantity only in regions, where enough bovine fetuses are accumulated at the slaughtering. This is only given in regions with extensive cattle husbandry.

FBS provided by Biochrom AG principally comes from South America and Australia. FBS from Biochrom AG is tested for endotoxins, mycoplasma, viral contamination and viral antibodies (against BVD-MD, BHV-1, PI3).

Which cells do you like to cultivate? Furthermore we offer pretested FBS lots suitable for different mammalian cells, amniotic fluid cells, and hybridoma cells. Australian FBS (some current batches)

was tested as suitable for murine embryonal stem cells.

Biochrom AG additionally offers FBS with specific pre-treatment:

- γ -irradiated FBS
On request, γ -irradiation is available for improved viral safety (irradiation dose ≥ 30 kGray)
- charcoal-stripped FBS, hormone-free (cat. no. S 3113, 100 ml)
- on request, heat-inactivated or dialyzed sera (exclusion limit 10 kDa or 12 – 14 kDa) (please enquire for conditions and prices)
- tetracyclin-free FBS

Do you need a new FBS sample?

Please fill in the form to request samples (see appendix) and send it to us via fax.

Or request samples online: fill in the form www.biochrom.de/products/sera/sample-order-fbs.

Product	Cat. No.	Unit
FBS, origin: EU-approved countries (South America) virus tested Storage temperature: -20°C	S 0113 S 0115	100 ml 500 ml
FBS, origin: EU-approved countries (Argentina) (with Certificate of Suitability, CoS; R1-CEP 2001-030), virus tested acc. to EMEA guidelines Storage temperature: -20°C	S 0213 S 0215 S 0210	100 ml 500 ml 1000 ml
FBS, origin: Australia (with Certificate of Suitability; CoS; R1-CEP 2001-032), virus tested acc. to EMEA guidelines Storage temperature: -20°C	S 0413 S 0415 S 0410	100 ml 500 ml 1000 ml

Biochrom AG FBS is:

- **Virologically tested** on Hoof and Mouth Disease, Bovine Rhinotracheitis (IBR), Bovine Parainfluenza (PI3), Bovine Diarrhoeovirus (BVD), and additionally if necessary on Blue Tongue Virus (BTV). FBS from Australia, and Argentina is even wider tested according to the EMEA Directive for Pharmaceutical Raw Material of Bovine Origin.
- **BSE-free** due to its origin: Biochrom AG principally buys and processes only raw sera from countries that have no original BSE cases according to the permanently updated list of the World Organization for Animal Health (OIE) in Paris.
- **Endotoxin-reduced** due to precise accumulating and clearing processes. Endotoxins are residues of the bacterial cell wall. It is very difficult even with the help of filtration processes to subsequently remove endotoxins from the sera.

Import of FBS

In the last years the worldwide FBS need increased permanently. However, the import and export of the serum is strictly regulated for the USA and the European Union. Though the European Union (EU) allows the import of South American serum ("EU approved"), while the USA makes it generally impossible, bio pharmaceutical producers in Europe are requested to use FBS from Australia or the US, in order to make the finished product worldwide marketable.

The EU currently allows the import of FBS under two alternative conditions:

- The raw serum can be imported without prior treatment, if it has its origin in the regions where the serum is veterinary-regulatory tested on the above-named viruses, and where no vaccination against these forms of viruses has been effected.
- Sera that are not certified according to the described instructions, need prior treatment whether gamma-irradiation of minimum 25 kGray, or alternatively heat treatment of 3 hours at +65 °C, or exposure to 3 hours pH-value-lowering onto 5.

It is incumbent upon the respective EU country that imports the serum first to control these import conditions.

The imported raw FBS from South America or Australia is delivered to Biochrom AG without interruption of the cold chain, and after assay and approval by the quality control, defrozen under standardized conditions, and sterile filtered with a multilevel filter-cascade. The usual processing lots have a standard volume of 1000 litres or 2000 litres for industrial customers. Before the filtrated serum is released for sales, it undergoes a sterility control according to the European Pharmacopoeia directives and a test on bovine viruses in an independent laboratory. Fillings of 100 ml for testing are available of each of these lots. The quality control results are reported in batch-related certificates that are provided together with the serum samples (request for samples see appendix).

Documentation

Especially if FBS is used in mayor quantities for productions ("bio-processing"), a complete chain of evidence in the documentation, from the origin to the sterile filtration, is condition to its acceptance. This is certified for FBS with particular origin with the Certificate of Suitability (CoS) issued by the EDQM in Strasbourg for Pharmaceutical Raw Materials of Bovine Origin. Together with the Sterility Quality Control Certificate, a mycoplasma-free testing and a successful pre-testing on standard-cell lines, the identification badge for the integrity and functionality of each lot is given. An independent laboratory additionally raises a series of clinically-chemical data provided to the customers together with the samples.

Storage and handling

FBS offers at moderate costs a complex mixture of valuable growth factors and trace elements for cell culture. The delicate texture of most of the components requires a cooling of the FBS of < -20 °C from the extracting of the raw serum to the use of the finished product. A correct handling of the cold chain preserves the FBS during a long storage and use. An unrestricted use of 5 years of the Biochrom AG serum is guaranteed under previously described conditions.

For some purposes, the sterile filtered serum may need further processing. For the biopharmaceutical use for example, a γ -irradiation on a minimum of 30 kGray is necessary for virus safety. In order to preserve an optimal efficiency regarding the benefit of the cell growth, freezing and de-freezing should result as quickly as possible to reduce the phenomenon of salt loss and to avoid the production of high salt concentrations (in not yet frozen parts of the serum) (see above "Sera").

SERA

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REAGENTSCELL CULTURE
DIAGNOSTICSCYTOKINES AND
GROWTH FACTORSMYCOPLASMA
REAGENTSCELL ATTACH-
MENT FACTORSINDUSTRIAL
CELL CULTURE

FBS Superior: Standardized serum

In order to even the usual variations of the biological characteristics of FBS batches, Biochrom AG offers with FBS Superior a standardized quality serum. Due to the addition of selected synthetic supplements, a continuous high level of quality is guaranteed.

Manage routine cell culture in the future without any need to test FBS batches, and use the FBS Superior that provides constant quality and excellent growth-enhancing characteristics.

- once tested, FBS provides a balanced constant growth profile
- save further testing in the future
- the costs for FBS Superior are the same compared to usual EU-approved FBS
- FBS Superior is always available
- virus tested
- mycoplasma-free

On the next testing, also ask for FBS Superior and convince yourself of the high quality and the benefits, which offers FBS Superior from Biochrom AG.

Product	Cat. No.	Unit
FBS Superior	S 0613	100 ml
Storage temperature: -20 °C	S 0615	500 ml

Other sera

On request, heat-inactivated or dialyzed (exclusion limit 10 kD or 12 - 14 kD) sera are available. Please enquire for prices and terms.

Analytical data for all sera are available on request, please enquire for prices and conditions.

Product	Cat. No.	Unit
Newborn Calf Serum (with CoS)	S 0123	100 ml
tested for mycoplasma, viral contamination, and viral antibodies (BVD-MD, BHV-1, PI3)	S 0125	500 ml
(with Certificate of Suitability, CoS; R1-CEP 2001-031)	S 0120	1000 ml
Storage temperature: -20 °C		
Porcine Serum	S 0163	100 ml
tested for mycoplasma		
Storage temperature: -20 °C		
Donor Horse Serum	S 9133	100 ml
tested for mycoplasma	S 9135	500 ml
Storage temperature: -20 °C		

CERTIFICATE OF ANALYSIS

Manufactured by BIOCHROM AG, Leonorenstr. 2–6, D-12247 Berlin

Phone: +49 30 7799060 – Fax: +49 30 7710012

Product: FETAL BOVINE SERUM (FBS), Origin Brazil
0.1 µm Sterile Filtered

Catalog No.: S 0113, S 0115, S 0110
Lot No.: 0141 L

Date of Production: 14.02.2007
Date of Approval: 19.03.2007
Expiry Date: 02/2012

Processed from raw material (Name or Number): 070053

Test	Units	Specification range	Results
Appearance, colour		clear, amber	complies
Sterility (Ph.Eur.)		No evidence of microbial growth	complies
pH (Ph.Eur.)		6.35 – 8.15	7.26
Osmolality (Ph.Eur.)	mOsmol kg/H ₂ O	280 – 340	310
Hemoglobin	mg/100 ml	< 50	26.72
Growth promotion performance	% of control	≥ 75 %	complies
Cloning efficiency	% of control	≥ 75 %	complies
Endotoxin (Ph.Eur.)	EU/ml	< 25	1.27

Virus testing

BVDV/MDV *)	not detected	complies
IBRV/BHV-1 *)	not detected	complies
PIV 3 *)	not detected	complies

Bovine virus antibodies against

BVDV/MDV *)	registration	< 2
BHV-1 *)	registration	< 2
PIV 3 *)	registration	< 2

Mycoplasma testing (PCR)

Mycoplasma **)	not detected	complies
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Tests performed at: *) Institute for Virology, Hannover, **) Minerva Biolabs, Berlin

CERTIFICATE OF ANALYSIS

Parameter/Biochemical Assay	Unit	Results
Alk. Phosphatase	U/l	394
GOT (AST)	U/l	47
GPT (ALT)	U/l	9
γ-GT	U/l	10
Bilirubin total	mg/100 ml	0.27
LDH	U/l	617
CK (NAC) total	U/l	139
Cholesterol	mg/100 ml	33
Creatinine	mg/100 ml	2.57
Urea	mg/100 ml	29

Electrolytes:

Sodium	mmol/l	142
Potassium	mmol/l	> 8.0
Calcium	mmol/l	2.83
Magnesium	mmol/l	1.10
Phosphate	mmol/l	2.45
Iron	μg/100 ml	147
Glucose	mg/100 ml	68
Protein	g/100 ml	3.6

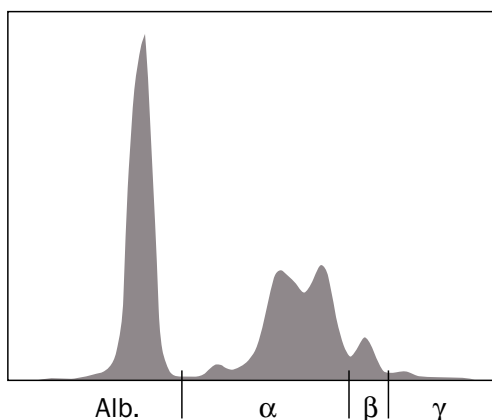
Elelectrophoresis:

Albumine absolute	g/100 ml	1.8
α-Globulin absolute	g/100 ml	1.6
β-Globulin absolute	g/100 ml	0.2
γ-Globulin absolute	g/100 ml	0.1
Alb./Globulin-Quotient		0.98

Hormones:

Estradiol	pg/ml	77.50
Progesterone	ng/ml	0.25
Testosterone	ng/ml	0.11

Electrophoretogram:



Country of origin of crude blood: Brazil
Country of serum processing: Germany

Storage: ≤ -20 °C

Antisera

Antisera against virus protein epitopes of HIV-1 and HIV-2, are produced by immunizing sheep with synthetic peptides. Antibodies are purified by means of affinity chromatography.

Serological activity, and specificity of the antibodies is checked using ELISA technique.

Product	Cat. No.	Unit
Antiserum against HIV-1, p24 Combination from three sheep-anti-HIV-1-p24 sera; lyophilized (gag) Storage temperature: +2 – +8 °C	D 7320	2.0 mg
Antiserum against HIV-1, gp120 Species: sheep, lyophilized (env) Storage temperature: +2 – +8 °C	D 7324	2.0 mg

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