2. Sera

Introduction 2.1
COS-Certificate .....2.2 - 2.3
Bovine Sera 2.3
Treated Sera .....2.4 - 2.5
Other Animal Sera 2.6
Sera Plus 2.6
Human Sera 2.7
Certificate of Analysis 2.8

Introduction

The function of serum in the cell culture

- To stimulate cell growth, to proliferate and differentiate through hormonal factors.
- Adhesion factors facilitate/enhance cell attachment on culture dishes (biomatrix).
- Transport and binding proteins provide hormones, minerals or lipides, among others.
- To bind toxic substances through serum proteins.

Animal serum

Irrespective of the cell line (= type or class of cell) a dose of serum (about 10 - 15 % of the total liquid volume) is added to the cell cultures as a nutrient.

Normally serum is produced from animal blood, and the so-called FBS is most widely spread out because it contains especially high growth rate factors due to its origin – the blood of foetuses left over in slaughted cattle.

Advantages of PAN Biotech GmbH

- Own raw material resources in different countries: Australia (US-approved), South Africa, South America (Brazil).
- Certificate of Suitability No.: R1-CEP 2002-167-Rev 00.
- Licensed according to the new EU-decree no. 1774/ 2002 with Vet. No.: DE 09 275 0001 14.
- Every single batch is completely documented – from the country of origin to the filtration. Our standard operating instructions can be requested any time.
- Each process, from collection of the raw serum till the sterile filtration of the end product are specified in SOP's (Standard Operating Procedures) and can always be allocated.
- We offer special types of serum: dialysed, heat-inactivated, carbon-absorbed, gamma-irradiated, delipidized, gamma-globulin reduced.
- Highest production and safety standards for serum production.
- Best references from industry and research.
- Very extensive analysis tests (see certificate).

Documentations

- COS-certificate
- Production report
- Certificate of analysis (CoA)
- Veterinary documents
- Import documents
- Certificate of origin
- Validation of the raw serum extraction and raw serum processing.
- Export-/Import documents according to the valid EUdirectrives.
- Production protocol

We exclusively use sera from guaranteed BSE-free collection areas. In addition, we can also confirm that South Africa, as a country of origin, is free from scrapie. No serum batches from PAN-Biotech is processed from Great Britain as the country of origin. We insist on the submission of a complete documentation, consisting of a certificate of origin and a veterinary certificate, shipping documents, a certificate of analysis and a detailed production protocol. Each operation during an individual production process is documented and then summarized in a production protocol.

Production process

The untreated serum is collected from selected slaughterhouses subjected to control checks. After the product is received, the serum is checked, pooled and then sterile filtrated through a series of filters of decreasing pore size. After decanting the serum into high-quality, sterile plastic/ nalgene bottles, comprehensive tests are carried out in the Quality Control Laboratory.

Sterility control

5 ml of each sample is inoculated in each case with 20 ml caso – and thioglycolate nutrient broth and incubated at 32 °C and at ambient temperature. Evaluation of the sterile control checks is completed after a period of 21 days.

Mycoplasma test

1. DNA-fluorochrome method:
The cell cultures are treated in accordance with the DNA fluorochrome method with H 33258 (bis-benzimide) and examined under a fluorescent microscope for the detection of mycoplasma contamination.

2. Microbial culture:
Sediment aliquots from the serum sample in an enrichment culture (biphasic broth with horse serum) are incubated for 14 days at 37 °C in the incubator. The enriched broth is then inoculated on agar plates. Assessment under the microscope is carried out after a 14 day incubation period under anaerobic conditions at 37 °C.

Bacterial L-forms

A special enrichment broth is used for the detection of bacterial L-forms.

Viral antigen-antibody test

All foetal calf sera are examined for virus contamination with BVD-MD, IBR-IPV and also with PI 3 micro-organisms in a recognised, independent test laboratory.

Cell growth

An aliquot is used as an additive to the growth medium (10 % serum) for the culturing of mouse fibroblasts (L 929) and mouse myeloma cells (SP 2). A growth curve is plotted over a 7-day period and an assessment is made of the division rate, cell count and cell morphology.

Clone formation capacity

500 cells are cultured in 90 mm tissue culture dishes and incubated for 14 days at 37 °C, after which the cells undergo fixation and staining. The following data are recorded: colony count, clone size and cell morphology. The serum is tested with L 929 for its plating efficiency and with SP 2 for its cloning efficiency. All the values determined in biological systems apply only to the indicated cell system. In addition to these routine studies we undertake further studies in different cell systems on request. We are pleased to comply with your wishes in this respect.

Clinical chemistry studies

Routine determinations such as glucose and total protein contents, pH value, osmolality, pyrogenicity, haemoglobin and further tests can of course also be carried out.

COS-Certificate

edqm
European Directorate for the
Quality of Medicines & HealthCare

COUNCIL OF EUROPE CONSEIL DE L'EUROPE

Certification of Substances Division

Certificate of suitability
No. R1-CEP 2002-167-Rev 00

Name of the substance:
FOETAL BOVINE SERUM

Name of holder:
PAN BIOTECH GMBH
Am Gewerbepark 13
Germany-94501 Aidenbach

Site(s) of production:
PAN BIOTECH GMBH
Am Gewerbepark 13
Germany-94501 Aidenbach

THIS CERTIFICATE SUPERSEDES THE PREVIOUS CERTIFICATE
R0-CEP 2002-167-REV 01

After examination of the information provided on the origin of raw material(s) and type of tissue(s) used and on the manufacturing process for this substance on the site(s) of production mentioned above, we certify that the substance **FOETAL BOVINE SERUM** meets the criteria described in the current version of the monograph Products with risk of transmitting agents of animal spongiform encephalopathies no. 1483 of the European Pharmacopoeia, current edition including supplements.

- country(ies) of origin of source materials: Australia and Brazil
- nature of animal tissues used in manufacture: Foetal bovine blood

The submitted dossier must be updated after any significant change that may alter the quality, safety or efficacy of the substance, or that may alter the risk of transmitting animal spongiform encephalopathy agents.

Manufacture of the substance shall take place in accordance with a suitable quality assurance system such as ISO 9001, and in accordance with the dossier submitted.

Failure to comply with these provisions will render this certificate void.

The certificate is valid provided there has been no deterioration in the TSE status of the country(ies) of origin of the source material.

Address: 7, allée Kastner, CS 30026 - F - 67081 Strasbourg (France)
Telephone: 33 (0) 3 88 41 30 30 - Fax: 33 (0) 3 88 41 27 71 - e-mail: cep@edqm.eu
Internet : http://www.edqm.eu

COS-Certificate

History:
PAN-Biotech GmbH was established in 1988 and has produced and sold animal serum products since 1994. The quality system for the manufacturing of these products is ISO 9001 certified.

(For the quality manual and ISO 9001 certification refer to the appendix 1 and 2)

The produced serum products have been used as raw materials for manufacturing biopharmaceutical products. The facilities and processes used for manufacturing are environmentally controlled and validated, and they guarantee sterile manufacturing.

From the procurement of the raw material serum to the finished product release, the processes of PAN-Biotech GmbH are designed to supply its customers with all the required documentation regarding the manufacturing processes and testing in order to satisfy their regulatory requirements.

Declaration:
The manufacturing process and quality control testing are done in accordance with the dossier presented and with a suitable quality assurance system in compliance with ISO 9001 quality standards. This quality assurance system verifies traceability and batch consistency. PAN-Biotech conducts internal and external audits of its quality system on an annual basis. Also, PAN-Biotech audits its raw material serum suppliers on a cyclical basis and reviews the facilities, manufacturing processes and documentation for the collection, handling, storage and transport of raw sera.

PAN-Biotech is willing to be inspected, in accordance with the relevant legislation, on the request of a relevant authority before and/or after being granted a certificate of suitability.

Aidenbach, 1st January 2011

Andreas Friedrich, Michael Wiechmann
Managing directors of PAN Biotech GmbH

Name of the holder/ PAN Biotech GmbH
Production site: Gewerbepark 13 94501 Aidenbach/Germany

Bovine Sera

COS: CEP 2002-167 Foetal bovine serum: South Africa Virus and mycoplasma tested, Health class 1a	100 ml 500 ml	1501-Lot# 1502-Lot#
COS: CEP 2002-167 Foetal bovine serum: South America Virus and mycoplasma tested, Health class 1a	100 ml 500 ml	3301-Lot# 3302-Lot#
COS: CEP 2002-167 Foetal bovine serum: AUSTRALIA US admissible Virus and mycoplasma tested	100 ml 500 ml	1301-Lot# 1302-Lot#
Foetal bovine serum: USA origin Virus and mycoplasma tested	100 ml 500 ml	1401-Lot# 1402-Lot#
Foetal bovine serum: US admissible Virus and mycoplasma tested (in accordance with the Aphis List)	100 ml 500 ml	1701-Lot# 1702-Lot#
Calf serum – newborn Virus and mycoplasma tested	100 ml 500 ml	0401-Lot# 0402-Lot#
Bovine serum Virus and mycoplasma tested	100 ml 500 ml	P30-0601 P30-0602

Treated Sera

Dialysed Serum

METHOD:
Serum is dialyzed with a 10,000 Dalton exclusion membrane against physiological saline solution (alternative: PBS) until the glucose content is below 10 mg/dl.

APPLICATION:

- Radioactive integration studies.
- Hormone-free applications.
- Tests intolerant of small molecules such as nucleotides (hypoxanthine, thymidine), amino acids (serine, alanine etc.), sugars or metabolites.

Foetal bovine serum dialyzed Virus and mycoplasma tested	100 ml 500 ml	P30-2101 P30-2102

Activated Charcoal Treated Serum

METHOD:
Serum is heated in a water bath with dextran and activated charcoal. The activated charcoal, together with the substances bound up in it, is then removed by means of centrifugation and filtration.

APPLICATION:

- Work involving reduced hormone content (steroids).
- Work involving reduced growth factors (prevention of cell differentiation).
- Receptor studies (e. g. oestrogens).
- Minimizes lot-to-lot variations in serum.

Foetal bovine serum activated charcoal absorbed Virus and mycoplasma tested	100 ml 500 ml	P30-2301 P30-2302

Heat inactivated Serum

METHOD:
Serum is heated for 30 min. to 56° C in a water bath,whereby it is shaken several times.

APPLICATION:

- Measurements of lactate dehydrogenase in the culture supernatant as a marker for cell damage (serum LDH is destroyed by heat inactivation).
- Minimizes lot-to-lot variations in serum (all thermolabile components are destroyed).
- Studies of vitamins and growth factors (are damaged wholly or partially, or have their concentrations reduced, by heat inactivation).
- Enhance viral safety, since heat-labile viruses are inactivated.
- Tests that do not tolerate presence of complement (complement destruction).

Foetal bovine serum heat inactivated Virus and mycoplasma tested	100 ml 500 ml	1901-Lot# 1902-Lot#

Tetracylin free Serum

METHOD:
Serum is tested for absence of tetracycline using the TET-off system (luciferase).

APPLICATION:

- TET-on / TET-off – regulated gene expression
- Transfections
- Expression studies

Foetal bovine serum tetracyclin free Virus and mycoplasma tested	100 ml 500 ml	P30-3601 P30-3602

Treated Sera

Delipidized Serum

METHOD:
Lipids are removed from serum by means of affinity chromatography.

APPLICATION:
- Lipometabolism studies
- Arteriosclerosis research

Foetal bovine serum delipidized	100 ml	P30-3401
Virus and mycoplasma tested	500 ml	P30-3402

Gamma irradiated Serum

METHOD:
Serum is exposed to irradiation with at least 25 kGy.

APPLICATION:
- Biopharmaceutical production
- Virus production
- Vaccine production
- Production of diagnostic items

Foetal bovine serum gamma irradiated	100 ml	P30-2001
Virus and mycoplasma tested	500 ml	P30-2002

Ultra low IgG Serum

METHOD:
IgG content (average in FBS approx. 190 mg/ml) is reduced by affinity chromatography (protein-G affinity column) to max. 5 mg/ml.

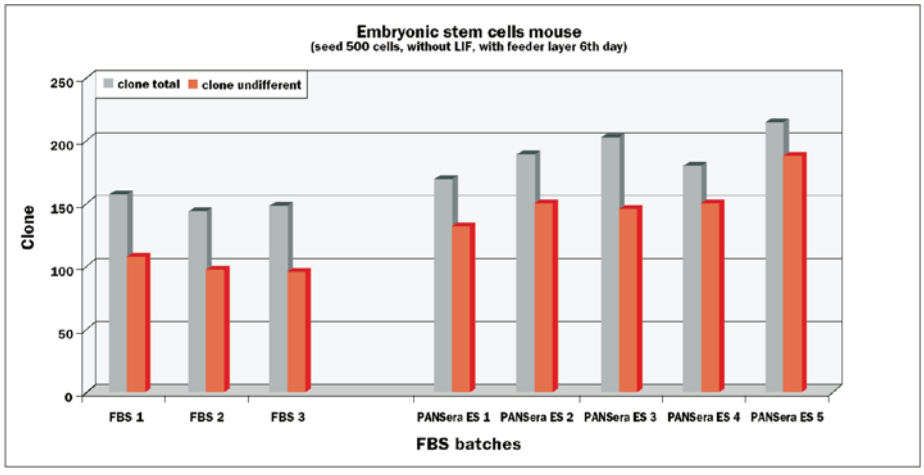
APPLICATION:
- Antibody production
- Monoclonal antibodies
- Radioactive markings

Foetal bovine serum ultra low IgG	100 ml	P30-2801
Virus and mycoplasma tested	500 ml	P30-2802

PANSera ES

Our new developed special processing procedure of Foetal Bovine Serum enables us, to offer you a SPECIALSERUM for embryonal stem cells.

ADVANTAGES:
- Reproducible, constant growth properties.
- Improved cloning efficiency.
- More undifferentiated clones.
- Permanent strict quality control.



Once PANSera ES, always PANSera ES.

PANSera ES FBS special designed for ES cells	100 ml	2601-Lot#
Virus and mycoplasma tested and pretested for ES cells	500 ml	2602-Lot#

Other Animal Sera

Chicken serum Virus and mycoplasma tested	100 ml 500 ml	P30-0301 P30-0302
Donkey serum Virus and mycoplasma tested	100 ml 500 ml	P30-0101 P30-0102
Goat serum Virus and mycoplasma tested	100 ml 500 ml	P30-1001 P30-1002
Hamster serum Virus and mycoplasma tested	10 ml	P30-0210
Horse serum Virus and mycoplasma tested	100 ml 500 ml	P30-0701 P30-0702
Lamb serum Virus and mycoplasma tested	100 ml 500 ml	P30-0801 P30-0802
Mouse serum Virus and mycoplasma tested	10 ml 100 ml	P30-0200 P30-0201
Pig serum Virus and mycoplasma tested	100 ml 500 ml	P30-0901 P30-0902
Rabbit serum Virus and mycoplasma tested	100 ml 500 ml	P30-1101 P30-1102
Rat serum Virus and mycoplasma tested	10 ml 100 ml	P30-01901 P30-01901E
Sheep serum Virus and mycoplasma tested	100 ml 500 ml	P30-4101 P30-4102

2

Sera Plus

For research purposes For the production of vaccines For the production of bioactive proteins (e. g. insulin) For the production of other protein molecules

Sera Plus – The Advantages

- Reproducible, constant growth properties
- Constant low endotoxin content
- Higher safety (viruses, mycoplasma)
- Several cell lines show better growth properties
- Suitable for a great variety of cells
- Permanent strict quality control

Reproducible, constant growth properties! Higher safety concerning the absence of viruses!

Sera Plus is a special processed serum. Serum of selected batches is separated into its individual components by a sophisticated chromatographic process, which are then added together again in a defined process. The aim was to produce serum with constant growth properties (defined composition) and with higher safety concerning the absence of viruses.

Examinations have shown that our special sera are superior in tests of customers. In comparison with normal FKS batches our special sera support the growth of many cell types equally well, often even better.

Sera Plus special processed FBS	100 ml 500 ml	P30-3701 P30-3702
Sera Plus S special processed FBS	100 ml 500 ml	P30-4801 P30-4802

Human Sera

Human Serum is manufactured from Human Plasma by adding calcium chloride. After removing the clot, the Human Serum is washed and concentrated by ultrafiltration and finally filtered through a combination of depth- and membrane-filters.

Please ask us especially for:

- Human Sera male
- Human Sera delipidized
- Human Sera AB
- Human Sera Sepharose A stripped
- Human Sera charcoal stripped
- Human Sera resin stripped

OFF THE CLOT SERA (True Human Sera)

Off the clot Serum is prepared from human whole blood collected without any coagulant, allowed to clot at room temperature and then centrifuged to remove the clot. We provide single donor units as well as pooled Off the clot Serum.

Off the clot Serum is filtered through depth-and membranefilters before filling.

Please ask us for single donations or pools of mixed genders, male, female or AB.

Human Serum	100 ml 500 ml	P30-2401 P30-2402
Human AB Serum	100 ml 500 ml	P30-2501 P30-2502
Human AB Serum (male)	100 ml 500 ml	P30-2901 P30-2902
Human Serum off the clot	100 ml 500 ml	P30-2701 P30-2702

Certificate of Analysis

CERTIFICATE of ANALYSIS

Product :	Foetal Bovine Serum	Cat.-No.:	3302-P330207
Lot-Nr :	P330207	Origin:	South America
Expiry:	05/2015		

Result:			Guide value	Result:			guide value :
pH:	7		6,5-8,0	Osmolality:	310	mOsmol/kg	280-350
Hemoglobin:	25,0	mg/100 ml	5-30	Total Protein:	3490	mg/100 ml	3200-4200
Sterility:	sterile		sterile	Endotoxin:	0,32	ng/ml	
Mycoplasma:	negative		negative	Glucose:	133,4	mg/100 ml	
Virus Testing:	negative		negative	IgG:	180	µg/ml	50-300
Albumin	2200	mg/100 ml	1800-3500	Cholesterol:	35,6	mg/100 ml	25-55
α-Globulin	890	mg/100 ml	500-2000	Triglycerides:	75	mg/100 ml	45-90
β-Globulin	400	mg/100 ml	300-900				

Cell Growth (number of cells per ml)

Cell line L 929	Seed	2. day	5. day	7. day
Test charge	1,00E+04	2,38E+04	7,00E+05	9,55E+05
Control	1,00E+04	2,55E+04	6,00E+05	9,10E+05

Cell line SP2	Seed	2. day	5. day	7. day
Test charge	1,00E+03	7,76E+03	8,20E+05	1,25E+06
Control	1,00E+03	6,94E+03	8,10E+05	1,14E+06

2

Plating Efficiency (L 929)

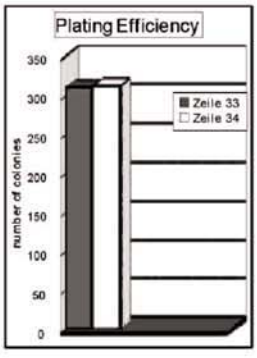
Generationtime L929:	sample	control
	14,71	**15,75 h**

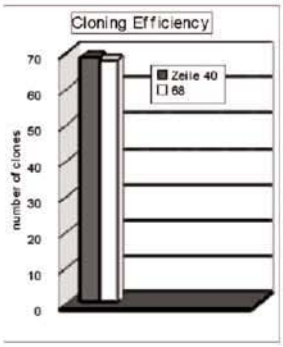
	Number of colonies	absolute PE %	relative PE %
Test charge	310	62	99
Control serum	312	62	100

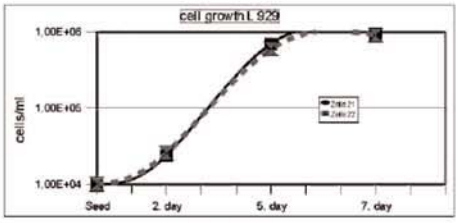
Cloning Efficiency (SP2)

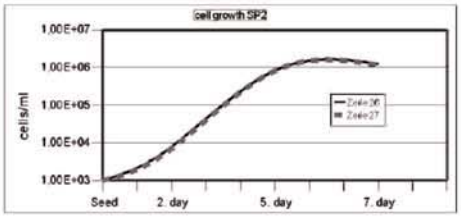
Generationtime SP2:	sample	control
	10,67	**10,45 h**

	Number of clones	absolute CE %	relative CE %
Test charge	69	72	101
Control serum	68	71	100









Aidenbach 27.01.2011

Dr Josef Seidl