

# Sera for Cell Culture

Quality, Consistency, Reliability



**Stand January 2016**

# Elite Biotech – Reliability in Serum

Elite Biotech is a German registered mark by miniBio GmbH founded in 2014 headquartered in University City Heidelberg and stands for premium biotechnology products at its birth.

Animal & human sera provide most of the growth factors, hormones and other cell stimulating factors required for successful cell culture. A core competence of Elite Biotech is the sourcing and manufacturing of sera products from several origins. Our sera portfolio comprises: Fetal bovine sera, bovine sera, a variety of other animal sera (horse, porcine, etc.) and human sera.

Elite Biotech has production facilities under contract in Europe and partners in USA and Australia. The European plant fulfils pharmaceutical standards and can manufacture under cGMP. Our serum manufacturing site is ISO certified.

Elite Biotech controls and documents all processes from raw material collection through to the final sterile filtered product, ensuring traceability from animal to the bottle. The supply chain follows our strict quality control standards and regular audits are performed to ensure all procedures are within our quality assurance standards. Constant monitoring of all processes ensures that only products of high quality and batch-to-batch consistency are manufactured by Elite Biotech.

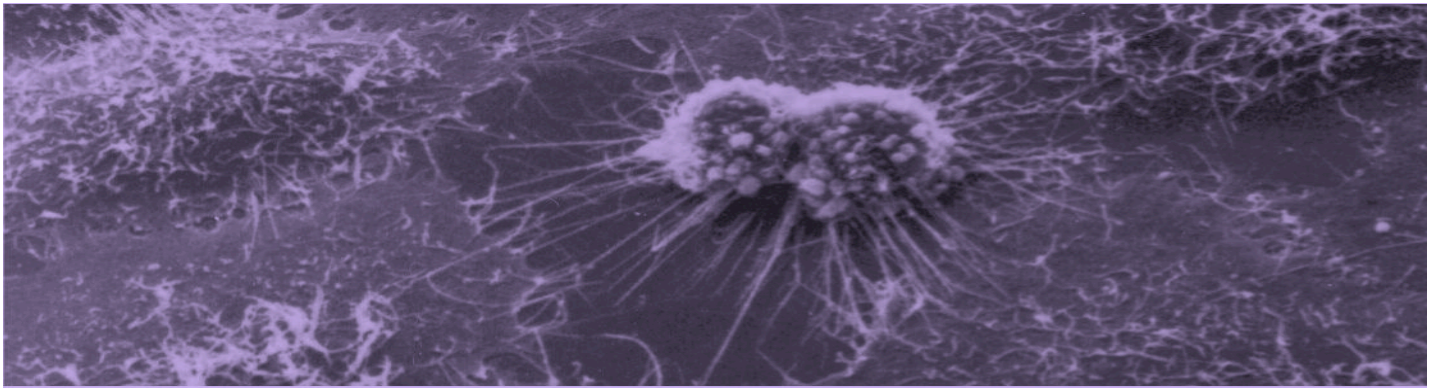
Extensive batch records including Certificates of Analysis (CoA) and other batch release documents are available for our customers.



**Fig 1:** Our modern production facilities guarantee the high quality of our serum products and help ensure the success of your research.

Elite Biotech offers much more than an excellent ordering and delivery service. On request, we are happy to supply samples of our serum products for you to try in your cell culture. The desired volume will be held on reserve for you until sample tests are completed. Normally, reservations are held for 6 weeks. You may advise Elite Biotech if longer reservation periods are required. For those customers who have placed an order but do not have sufficient storage space Elite Biotech can store their serum in controlled freezer rooms for future “call off” or “sold and storage” deliveries at desired times and quantities. On request, we can also supply serum in special packaging other than the standard 100 ml and 500 ml containers. Special treatments such as gamma irradiation, heat inactivation, UV irradiation, dialysis, charcoal stripping are also part of our service as well as specific animal virus and other pre-testing. Just give us a call, we will be happy to advise you.

Our aim is not only to manufacture high quality products for your cell culture, but also to support you with documentation and practical assistance in your day to day scientific work. For this purpose Elite Biotech has a technical service team with the skills to provide you with further assistance in matters relating to cell culture. Should you need further information about sera or other Elite Biotech products, details on composition and physiochemical properties can be found in our main catalog or in the internet under [www.elite-bitech.com](http://www.elite-bitech.com).



**Fig 2:** The addition of serum to culture medium ensures a natural cell cycle and metabolism.

## Serum – Still the ‘Elixir’ of Cell Culture Life

Since the early 20th century scientists have been searching for methods to allow the isolation and growth of tissues and cells outside the body in what we now term as in vitro conditions. In the late 1940's the first cell line (HeLa) was cultivated in vitro in a fluid mixture of chicken plasma, bovine embryo extract and serum from umbilical cord blood. This crude mixture was the fore runner of today's modern cell culture media.

The ideal culture medium simulates the exact in vivo conditions. In practice this can only be achieved to a limited extent as the physiological conditions required to cultivate tissues, cells and living organisms are extremely complex.

Serum provides all of the growth factors, vitamins, co-factors, hormones, attachment factors (fibronectin, laminin), transport factors (albumin, globulin, transferrin), nutrients (nucleosides, amino acids, fatty acids, lipids), trace elements and other factors which limit free radicals, toxins and heavy metals. Serum is a very complex product and its components have not yet been fully identified.

Serum still remains the most effective growth product for cell culture available today.

Most sera used in cell culture are of animal, mainly bovine origin. The use of animal sera brings some disadvantages such as possible presence of antibodies which may impair or damage cell growth, possible presence of adventitious animal viruses and risk of contamination with endotoxins and mycoplasma which can damage fragile cell lines.

However, special techniques used in the collection of the raw blood as well as sophisticated production and filtration techniques have resulted in much higher quality sera being now offered to cell culture scientists.

From raw material collection through to final finished product Elite Biotech production facilities ensure that only sera of high quality and levels of safety are manufactured.

The 1980s and 1990s saw the development of serum-free media, however the use of sera still remains an essential part of both research and biopharmaceutical/vaccine production.

# Animal Sera – Origin

Since the first case of Bovine Spongiform Encephalopathy (BSE or Mad Cow Disease) in the UK in the mid 1980's, the safety requirements for the use of animal sera have become very stringent. However, it must be stressed that bovine sera, and in particular, Fetal Bovine Serum (FBS), are classified as 'low risk products' as far as Transmissible Spongiform Encephalopathies (TSE) are concerned. The causative agent of BSE is closely linked to brain and spinal cord tissues of bovines classified as specific risk materials that are removed and destroyed immediately.

Besides BSE a number of animal viruses may be present in animal sera. Due to the placenta barrier Fetal Bovine Serum is a product with lower level of potential viral contaminants compared to serum of older bovine animals. Diseases such as Foot and Mouth (FMD), Rabies, Herpes and others can all affect cattle worldwide.

Elite Biotech only selects raw serum from controlled sources and from countries approved by the official veterinary health authorities in the US, Australia, and the EU. We offer Fetal Bovine Serum under the following classifications:

**EU approved:** Fetal Bovine Serum manufactured from raw materials sourced mainly from Latin American countries such as Brazil, Chile, Argentina or other countries approved to export bovine blood products to the European Union.

**USDA approved:** Sera manufactured from raw materials sourced from North and Central American countries such as Mexico and Panama or other countries approved by the USDA to export bovine blood products to the US.

Elite Biotech also provides sera sourced exclusively from **USA** and **Australia**.

The European Union (EU) will only permit the import of bovine products, including blood and sera, from countries meeting specific criteria like disease status or absence of animal treatment with certain growth promoting factors prohibited by the EU. These criteria are met by materials from USA, Canada, Australia, New Zealand and certain countries in Central and Latin America.

The EU approved category of Elite Biotech's Fetal Bovine Serum and products manufactured therefrom can be freely moved between all EU member states and are eligible for exportation to other countries outside of Europe depending on the current requirements of the importing country.

The main authority responsible for importation, manufacturing and export of animal by-products in the USA is the United States Department of Agriculture (USDA). Serum and finished products manufactured from sera of US, Australian, New Zealand and other USDA approved origins, will be allowed to enter into the US if processed in a country acceptable to the USDA.

USDA approved serum, if processed in Europe, may not be shipped back to the US due to the risk of cross contamination with serum of origins not acceptable to the USDA. This is a general rule and special conditions may apply in case of research or other projects with US based laboratories.

In order to meet the requirements of all customers around the globe and also comply with the regulatory requirements in many different countries Elite Biotech offers FBS and other animal sera manufactured in its sites located in Europe, Australia and North America.



## Animal Sera – Collection

Most animal serum including FBS is a by-product of the meat processing industry. No animal is killed only to collect the serum. Blood is collected in an abattoir and the red blood cells and fibrin are separated from the serum. The raw serum is then frozen and shipped to our production facilities for further processing.

In the case of Fetal Bovine Serum skilled operators remove the blood from the fetus by aseptic cardiac puncture. This process is carried out in special areas within the abattoir using techniques to reduce the possibility of bacterial contamination which may result in presence of endotoxin in the serum. Other bovine and animal species' blood is collected in the abattoir using different techniques however under the same rules for prevention from bacterial and microbial contamination.

The raw serum is carefully centrifuged to separate the red blood cells and fibrin from the serum, pooled and frozen. Blood collection and processing are performed in a way to obtain raw serum with low levels of hemoglobin. Samples from the crude serum are tested for endotoxin and hemoglobin and the product meeting Elite Biotech's internal specifications is selected. Upon approval the serum is shipped to Elite Biotech's processing facilities.

Donor sera are collected by venipuncture from living animals. All donor animals are kept in controlled and certified donor herds, regularly checked by official veterinarians. Any animal showing signs of sickness is immediately isolated and placed into quarantine. All animals in the donor herds are subject to controlled feeding diets to ensure high quality and to contribute to good growth promoting properties of the serum.

All raw sera are processed and sterile filtered in modern production facilities working under Elite Biotech's supervision.

Upon arrival at the facility the raw serum is thawed, transferred to a pooling tank and then mixed to create a true pool. Following that, the serum is passed through a cascade of filters and bottled in our class 100 clean room environment. After bottling, the product is labeled with full product code, batch number and other details as required. The finished sterile filtered product is taken to a rapid cooling freezer and stored at -20 °C in our carefully controlled freezer rooms.



**Fig 3:** Each individual batch of serum is subject to an exhaustive program of testing.

Elite Biotech's production processes have been validated and are documented at each stage from the raw material collection through the whole production cycle. Upon customer's request this batch documentation can be provided.

Each batch of sera is extensively tested by our QC department. Full details of specifications and test results are available on the corresponding Certificate of Analysis.

All FBS products are packaged in gamma irradiated 100 & 500 ml PET bottles. Packaging in alternative containers and volumes is also available upon request.

Elite Biotech offers many additional treatments for FBS and other sera listed in the following pages.

# Animal Sera – Further Processing

Numerous innovative techniques and methods are used in the modern biopharmaceutical research and production. Many of these specific requirements on sera are used in cell culture. For this purpose some of our serum is post-treated in order to become particularly suitable for specific applications. Some of these treatments eliminate viruses and other microorganisms, thus increasing the biological safety of the sera. Other treatments remove various natural serum constituents which disrupt certain analytical techniques or assays.

Furthermore, we carry out tests to identify batches of serum with characteristics which make them well suitable for specific applications or certain cell lines.

## Heat inactivation

Before the advent of FBS, cells were cultivated in more adult sera containing high levels of components which could affect cell growth such as antibodies, complement factors and animal viruses.

To remove these components and viruses many laboratories used a Heat Inactivation (HI) process. The use of Fetal Bovine Serum together with vastly improved serum production processes has now, to a large extent, removed the need to heat inactivate serum. For other, mostly adult sera, heat inactivation may still be required.

### Tip

If you need to heat inactivate your serum then a carefully controlled process must be followed in order to avoid damaging its growth promoting properties and reducing the formation of unwanted precipitates.

The process involves heating the serum in a shaking water bath at exactly 56 °C for 30 minutes. The shaking will help avoid the formation of protein and other forms of precipitates. After 30 minutes the serum is cooled down to room temperature as quickly as possible to avoid excessive exposure to heat which can damage growth factors, vitamins, etc. The heat inactivation process will inactivate the complement system, antibodies and other active enzymes such as lactate dehydrogenase (LDH). It is therefore recommended to use heat inactivated serum for immuno-assays or if LDH is to be measured in the cell culture supernatant as an indicator of cell damage.

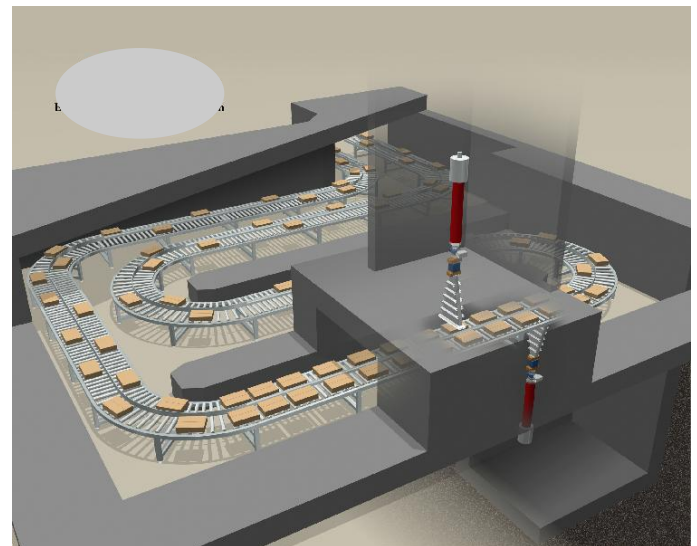
## Gamma irradiation

Sterile filtration can ensure that sera are largely free of micro-organisms; however, more stringent serum safety requirements apply to applications such as vaccine production. Such requirements are met by gamma irradiation with cobalt 60 ( $^{60}\text{Co}$ ) which further reduces or eliminates the viruses and mycoplasmas which may remain in the serum after filtration.

Serum is commonly irradiated at 25 kGy, for certain applications a dosage of 35 kGy is required.

The bottles of serum are generally irradiated on pallets. In order to achieve a dosage of 35 kGy in the center of a pallet, it must be irradiated with a total dosage of 50 kGy. The increased irradiation level generates extra heat which can result in damage to the serum, particularly in the outer boxes. This non-uniform irradiation of the bottles may impair the homogeneity of the batches of serum.

In order to achieve a more even distribution of the gamma irradiation dosage Elite Biotech has validated pallet irradiation for various fill sizes. Additionally, Elite Biotech has the possibility for single box irradiation. In this process, small packaging units are continuously conveyed past the radiation source. Each individual bottle of serum is exposed to a dosage of 35 kGy to at most 38.7 kGy. The sensitive growth factors are protected and the homogeneity of serum batches is retained.



**Fig 4:** The single box irradiation plant dedicated for sensitive liquids in Seibersdorf, Austria.

### Charcoal stripping

During the stripping process the serum is filtered through charcoal to remove non-polar material such as estradiol, progesterone, cortisol, testosterone, T3, T4 and insulin. Furthermore, some growth factors and cytokines are removed resulting in a suppressed cell differentiation. The concentration of salts within the serum is not affected. Charcoal stripping does not entirely eliminate the content of the steroid hormones but minimizes lot to lot serum variability.

### Dialysis

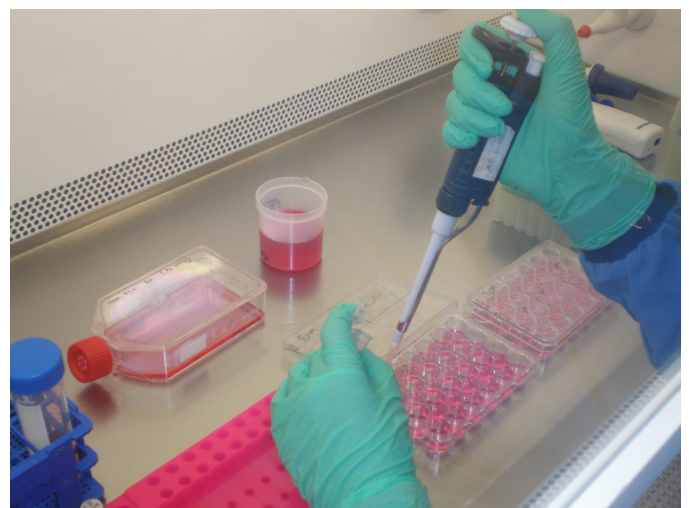
This process induces a distinct reduction in the serum's content of molecules with a molecular weight of  $< 10$  kDa, for example nucleotides and amino acids. This involves dialyzing the serum against a 0.15 M physiological saline solution through a membrane with a cut-off of 10 kDa. Dialyzed serum is suitable for use within incorporation studies, in which the addition of labelled nucleotides or amino acids is intended to reveal information about biochemical processes in the cell. The molecules contained in conventional serum would compete with the labelled molecules and so jeopardize the success of these studies. Various hormones are also removed from the serum by dialysis. This is an important factor in many receptor studies, as there is a risk that hormones from the serum will displace the test substances from the binding sites of the corresponding receptors.

### Pre-testing

Pre-testing identifies batches of serum which are particularly suitable for specific cell cultures because of their natural, individual characteristics.

Serum with low endotoxin content can be identified and provided upon request. The low endotoxin content is particularly favorable for culturing primary or hybridoma cells. We can also supply sera which are largely free of tetracycline or tetracycline derivatives ( $\leq 0.5$   $\mu\text{g/ml}$ ) and are so ideally suitable for use in Tet-On/Off gene expression systems.

Elite Biotech provides sera which testing has revealed to be particularly suitable for culturing embryonic stem cells (ES cells) or amnion and chorionic villi cells in cytogenetics.



**Fig 5:** Pretesting of low endotoxin batches of FBS for ES cell stimulation in specific assays.

# Elite Biotech's Serum Portfolio

## Fetal Bovine Serum Premium

Fetal Bovine Serum Premium is perfectly suitable for standard cell culture applications in academic research as well as in pharmaceutical production. It is produced by selecting raw material of premium quality and altering certain parameters to promote optimal cell growth with low batch-to-batch variation. Our FBS is available from different origins as well as with additional treatments, such as heat inactivation, gamma irradiation, etc. (upon request).

## Fetal Bovine Serum Superior

Raw FBS is a natural product obtained from bovine blood, showing remarkable batch variations due to various environmental influences, such as nutrition or health state of animals. Batch-to-batch variations of FBS may have major impacts on the reproducibility of scientific results.

Based on premium serum quality, Elite Biotech further develops FBS Superior through sophisticated treatments during pooling and filtration, which significantly reduces batch-to-batch variation and meanwhile also minimizes endotoxin level to enhance the stability of the serum as well as the proliferation of many different cell types.

FBS Superior is ideal for scientific research, calibration assays, fermentation and/or as stabilizer for diagnostic kits.

- Minimized batch-to-batch variation
- After initial testing no further batch testing necessary
- No batch reservation necessary
- Contains no other animal sera / by-products
- Suitable for all cell lines

### Fetal Bovine Serum Premium

Collected in South America	100 ml	FBS-Premium-S
	500 ml	FBS-Premium-L

Collected in Australia	100 ml	FBS-AUS-S
	500 ml	FBS-AUS-L

Collected in United States	100 ml	FBS-USA-S
	500 ml	FBS-USA-L

### Fetal Bovine Serum Premium, Heat Inactivated

Collected in South America	100 ml	FBS-Premium-HI-S
	500 ml	FBS-Premium-HI-L

Collected in Australia	100 ml	FBS-AUS-HI-S
	500 ml	FBS-AUS-HI-L

Collected in United States	100 ml	FBS-USA-HI-S
	500 ml	FBS-USA-HI-L

## Special Fetal Bovine Sera

Elite Biotech has in its portfolio a wide range of special Fetal Bovine Sera. Due to various treatments, sera tailored to specific biotechnology applications can be supplied. We offer special processes which enhance the level of safety with regard to contamination with viruses or other microorganisms.

Others serve to remove certain constituents from the sera which have a disruptive action in specific analytical methods. We can also identify batches of serum which are particularly suitable for specific applications because of their individual, natural characteristics.

## Fetal Bovine Serum, Heat Inactivated

Heat inactivates the complement system and many enzymes in this serum which has thus proved to be particularly valuable in immunoassays or certain enzyme tests. However, it is also suitable for all conventional applications, such as a culture medium additive, as a stabilizer or for coating culture vessels.

- Stabilizer
- For immunoassays
- Ideal for enzyme tests

### Fetal Bovine Serum Superior

Collected in South America	100 ml	FBS-Superior-S
	500 ml	FBS-Superior-L

Collected in Australia	100 ml	FBS-AUS-Superior-S
	500 ml	FBS-AUS-Superior-L

### Fetal Bovine Serum Superior, Heat Inactivated

Collected in South America	100 ml	FBS-Superior-HI-S
	500 ml	FBS-Superior-HI-L

Collected in Australia	100 ml	FBS-AUS-Superior-HI-S
	500 ml	FBS-AUS-Superior-HI-L



### **Fetal Bovine Serum, Gamma irradiated**

Gamma irradiation further reduces or eliminates viruses and mycoplasmas and so enhances the biological safety of the sera. This treatment is particularly suitable for the production of biopharmaceutical and diagnostic products, including virus and vaccine production.

- For biopharmaceutical production processes
- For the production of diagnostic products
- The safe choice for virus and vaccine production

### **Fetal Bovine Serum, Charcoal stripped**

Charcoal stripped FBS is used by researchers requiring low levels of hormones in the serum which may interfere with the experimental work.

- Investigation of effects of steroid hormones in vitro
- Receptor studies
- Ideal for immunoassays
- Provides low level of proteins
- Tested for cloning efficiency

### **Fetal Bovine Serum, Dialyzed**

Dialysis removes many hormones from this serum. The content of nucleotides and amino acids is also distinctly reduced. These properties make dialyzed serum specifically suitable for use in incorporation or receptor studies. Dialysis is performed with a cut off of 10 kDa, against a 0.15 M physiological saline solution. The process is performed until the glucose content is lower than 10 mg/dl.

- For radioactive incorporation studies
- For hormone reduced cell culture

### **Fetal Bovine Serum, ES Cell Pretested, USDA Approved**

Embryonic stem (ES) cells are particularly demanding and it is crucial to keep them in their valuable undifferentiated state. When working with stem cells maintenance of pluripotency and reduced differentiation are the main objectives.

Historically, EC cell researchers were required to screen laboriously several lots of serum to find one suitable for their cells. Elite Biotech offers ES Cell Pretested FBS to minimize the need for prescreening serum, saving both labor and cost.

The screening includes growth promotion, colony morphology, and cytotoxicity. ES Cell Pretested FBS is selected from our highest quality FBS lots.

#### Features

- Receptor studies
- Tested on murine embryonic stem cells
- Selected from highest quality FBS lots
- Strict quality control
- Consistent quality

### **Fetal Bovine Serum, Gamma irradiated**

Collected in South America	100 ml	FBS-GI-S
	500 ml	FBS-GI-L

### **Fetal Bovine Serum, Charcoal stripped**

Collected in South America	100 ml	FBS-CS-S
	500 ml	FBS-CS-L

### **Fetal Bovine Serum, Dialyzed**

Collected in South America	100 ml	FBS-DL-S
	500 ml	FBS-DL-L

### **Fetal Bovine Serum, ES Cell Pretested**

Collected in South America	100 ml	FBS-ES-S
	500 ml	FBS-ES-L
Collected in USA	100 ml	FBS-USA-ES-S
	500 ml	FBS-USA-ES-L

# Bovine Sera

For many cell cultures, Fetal Bovine Serum is an ideal additive to the medium as it contains particularly high levels of growth factors. The disadvantage is its relatively high price. Robust cell lines may also be cultured with other, lower cost bovine sera. As a rule of thumb, the older the animals from which the serum was obtained, the lower the growth promoting action and the greater the content of antibodies and protein. However, more moderate growth promotion than the one provided by FBS may be advantageous if there is a risk that, in the event of great multiplication, the cells will have to be passaged too frequently.



## Bovine Sera

Serum of this category originates from cattle more than 12 months old. It is not only used as an addition to cell culture media, but also as a control standard and for many assays, for calibrating measurement methods or for reducing non-specific reactions in ELISA.

## Donor Bovine Sera

Donor Bovine Serum is obtained from donor animals and not from slaughtered animals. It is usually sourced from controlled herds specifically tested on the absence of certain viruses and antibodies. Advantages over conventional Bovine Serum from slaughtered animals are the lower endotoxin and hemoglobin content and the lower level of batch-to-batch variation. Otherwise, it is used for the same applications.

## **NEW** Fetal Grow Serum

Elite Biotech's Fetal Grow Serum is newly developed by combining selected calf serum with naturally fortifying supplements of highest quality. Fetal Grow Serum has the advantages of minimized batch-to-batch variation and en-

hanced growth-promoting property, thus is suitable for vast applications.

## Newborn Calf Sera

Serum of this category originates from 3 to 10 days old calves and contains a higher proportion of proteins and immuno-globulins and fewer growth factors than Fetal Bovine Serum. Nevertheless, it is suitable for culturing most mammalian cell lines. Due to its lower price it is largely used in manufacturing, for example in fermentation processes.

## Calf Sera

Calf Serum is obtained from animals not more than 12 months old and its protein content is significantly higher than FBS. It is suitable for robust cell lines and short-term cell cultures, which do not need to be passaged many times, as it contains fewer growth factors than FBS or serum from newborn calves. Calf Serum is a lower cost alternative to FBS and is ideally suitable for fermentations. Furthermore, it can act as a stabilizer in diagnostic assays, as a control standard or for isolating bovine proteins.

## Donor Calf Sera

Donor Calf Serum does not originate from slaughtered animals but from donor calves bred in controlled herds specifically tested on the absence of certain viruses and antibodies. It is suitable for the same applications as conventional calf serum. However, it has much lower endotoxin and hemoglobin content than serum from slaughtered animals and exhibits lower batch-to-batch variations.

## Newborn Calf Serum

Collected in Australia/USA	100 ml	NCS-S
	500 ml	NCS-L

## Fetal Grow Serum

Collected in South America	100 ml	FGS-S
	500 ml	FGS-L

## Calf Serum

Collected in Australia/USA	100 ml	CS-S
	500 ml	CS-L

## Donor Calf Serum

Collected in Australia/USA	100 ml	DCS-S
	500 ml	DCS-L

## Adult Bovine Serum

Collected in Australia/USA	100 ml	ABS-S
	500 ml	ABS-L

# Human Sera

Human Serum in the culture medium provides human cell lines with an environment more closely matching their physiological conditions than media with animal sera.

Human serum may be superior to animal sera when culturing sensitive human cell lines. This particularly applies to immune cells such as macrophages or lymphocytes.

Human Serum is a highly sensitive material and is subject to the most stringent safety regulations. Elite Biotech's sera are obtained from blood and plasmapheresis centers in the US and the EU, approved and controlled by the national health authorities.



## Human Serum, Converted

Converted Human Serum is suitable not only as an additive to culture media but also for control assays, immunoassays, clinical chemistry and in vitro diagnostics. Elite Biotech can also supply converted human serum originating exclusively from donors of blood group AB whose blood contains neither anti-A nor anti-B antibodies which can damage cultured human cells and disrupt immunological investigations.

Converted Serum is produced from plasma, which still contains all the clotting factors and its composition largely corresponds to the physiological conditions in blood.

**Human serum is intended for research and diagnostic only, NOT for ex vivo or in vivo therapeutic use!**

**Human Serum, converted, Type AB**      100 ml    HUM

# Other Animal Sera

Other animal sera are used in various applications, e.g. autologous cell culture systems or immunohistological methods.

Porcine serum, for instance, is often used in cultures that proliferate mycoplasma or viruses. Goat and sheep sera are widely found in immunohistochemistry and/or to eliminate non-specific binding reactions.

## Applications:

- Autologous cell culture systems
- Immunoblotting
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation

<b>Horse Serum</b>	100 ml	HOS-S
	500 ml	HOS-L
<b>Donor Horse Serum</b>	100 ml	DHS-S
	500 ml	DHS-L
<b>Goat Serum</b>	100 ml	GOA-S
	500 ml	GOA-L
<b>Porcine Serum</b>	100 ml	POR-S
	500 ml	POR-L
<b>Mouse Serum</b>	100 ml	MOU-S
<b>Rat Serum</b>	100 ml	RAT-S
<b>Rabbit Serum</b>	100 ml	RAB-S

## Sera – Practical Tips

Sera are highly complex biological materials. Their biological activity depends on many factors such as correct handling during storage, freezing and thawing. Since water freezes first during the freezing process and ice floats to the surface due to its lower density, the concentrations of salt and other substances increase in the remaining liquid fraction. Therefore slow freezing may damage sensitive serum constituents. Quick freezing minimizes these effects and therefore Elite Biotech's sera are rapidly frozen after production.

### Storage

Elite Biotech's serum is supplied deep frozen in unbreakable PET bottles in a polystyrene foam package. If not shipped by a freezer truck it is delivered on dry ice. Any serum not used straight away should be stored protected from light at -15 °C to -40 °C. Partial thawing of the serum before it is placed into storage should be avoided. In the event of a partial thawing thaw the serum completely in a refrigerator, shake it and refreeze. Please avoid repeated thaw/freeze cycles. For short term usage, store serum in the refrigerator at +2 °C to +8 °C. If you require only small quantities of serum at a time, we recommend freezing small aliquots.

### Thawing

Thawing of sera should proceed as quickly as possible in order to minimize the period of time during which elevated salt concentrations prevail in the thawed liquid. The serum must not be exposed to high temperatures during thawing as sensitive molecules could be denatured. Bottles taken from the freezer should be allowed to acclimatize for approximately 10 minutes at room temperature or overnight in a refrigerator. The sera may then be thawed in a water bath at +37 °C. Make sure that the bottles do not float and tip over. Frequent swirling of the serum is important during thawing as this disperses the released salts and proteins uniformly in the liquid. Avoid repeated freeze-thaw cycles as precipitation may otherwise easily occur. The serum should be swirled occasionally while working at room temperature in order to ensure that the liquid remains homogeneous.

### Shelf Life

Serum may be stored  $\leq -15$  °C to -40 °C for five years without any appreciable reduction in biological activity. Elite Biotech has performed studies on FBS proving that all standard parameters are still within the specifications during the storage time of 7 days to either cooled or room temperature conditions. It was proven as well that 24 hours at conditions of 40 °C do not negatively harm the quality of FBS or alter single parameters significantly.

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