Fetal Bovine Serum, Embryonic Stem Cells tested

CAT N°: SxxxS

Collected from the source:
When searchers choose their serum an important factor that should be taken into consideration is the source, which also emphasises the traceability of the serum.
Our system of vertical integration allows us to be certain of the origins and traceability of our FBS.
Each manufactured batch is rigorously controlled, from the collection of serum and throughout all stages of its treatment and production through to final packaging on our premises.
BioWest Fetal Bovine Serum is derived from clotted whole blood aseptically collected from foetus via cardiac puncture.
The serum is collected or imported and treated in agreement with the European regulations.

Country of Origin:
The country in which the serum was taken from the donor/animal.
To see the countries of origin we can offer, please refer to the technical data sheet for the standard Fetal Bovine Serum (ref. FT.FBSan).

To order the treated serum, please replace the last number of the Cat N° of standard serum (written on the FT.FBSan) by the letter S.

Storage conditions:
- 18°C to - 40°C, protected from light.
Bottles can be stored between -40°C and -80°C for a short period (few days).

Shelf life:
5 years

Filtration:
Final Filter Size: 0.1µm x 3

pH:
pH specification: 7,4 ± 0,6

Osmolality:
Determined by a lowered freezing temperature. The osmometer is calibrated against standard solutions.
Osmolality specification: 322,5 ± 42,5 mOsm/kg

Endotoxin:
All sera are tested to determine the levels of endotoxins. BioWest carries out a chromokinetic quantitative test, according to the method D of the European Pharmacopoeia.
The endotoxin reagent is standardized against the US reference endotoxin.
Specifications available on Certificate of Analysis

Haemoglobin:
The haemoglobin level is measured by spectrophotometer.
Specifications available on Certificate of Analysis

Cell Culture:
Biological performance is assessed using cell culture medium supplemented with the serum being tested. During the test period, cultures are examined microscopically for any morphological abnormalities that may indicate toxic components in the serum.

Cell Culture Tests:
Cell Growth, Plating Efficiency, Cloning Efficiency.

Cell Lines Tested:
The following cell lines are tested with the serum:

HELA -Cancer Cell/Human.
L929 -Fibroblast-Mouse/ As Macrophage
SP2/O-AG14 -Mouse/Lymphoma
MRC- 5 -Human/Lung.

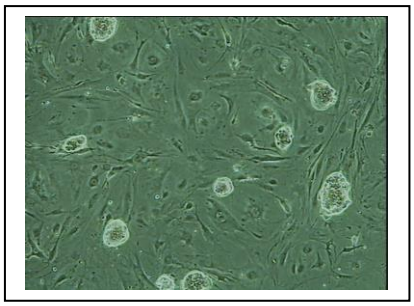
Total Protein:
Determined by Biuret Colorimetry.
Total Protein specification: 40 ± 15 g/l

Sterility tests:
All sera are tested for the absence of aerobic and anaerobic bacteria, fungi, yeast and *Mycoplasma*.
The sterility test is based on the European Pharmacopoeia requirements.
The sera are tested for the absence of *Mycoplasma* by culture.

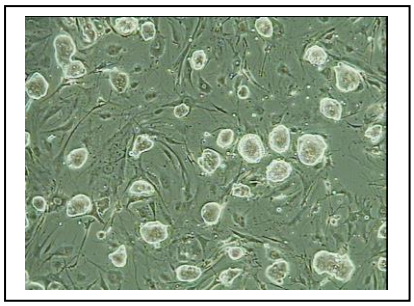
Virus test:
All of our sera are tested for:
- Bovine Viral Diarrhoea (BVD)
- Cytopathogenic agents e.g. Infectious Bovine Rhinotracheitis (IBR) / BHV-1
- Hemadsorbing agents e.g. Parainfluenza Type 3 (Pl3)

Sera are tested by inoculation to permissive cells. The revelation is made by immunofluorescence for pestiviruses. Cytopathogenic agents and hemadsorbing agents are detected by microscopic observations.

Other tests:
Embryonic Stem Cells tests:
BioWest delegates this test to an external laboratory.
The cell growth is studied during two passages with mouse embryonic stem cell E14 cell line.
The validation criteria are the cell growth and the morphology of the cells (see below photos of embryonic stem cells grown up on a BioWest fetal Bovine serum).

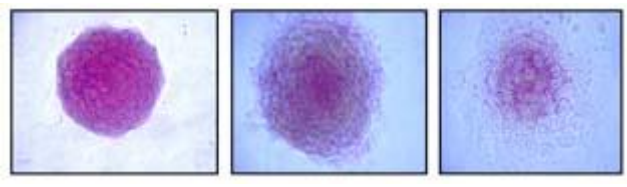


Serum at 10%, Passage 1, 72h



Serum at 10%, Passage 2, 72h

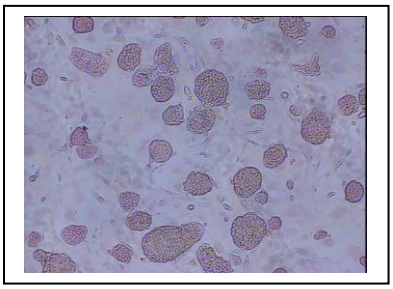
A validation of the cell growth test is made with the use of an Alkaline Phosphatase Detection kit. The Kit is a specific and sensitive tool for the phenotypic assessment of ES cell differentiation by the determination of the AP activity



A B C

Alkaline Phosphatase staining of ES cells. High magnification revealed :
-(A) Undifferentiated ES cells (mouse MBL.5 cell line)
-(B) Differentiated ES cells
-(C) Differentiated ES cells

See below a photo of embryonic stem cells grown up on a BioWest fetal bovine serum and revealed by the Alkaline Phosphatase Detection Kit.



A toxicity test is also performed with the serum at 30%.

Treatments:
Not Applicable

Recommended use:
- Respect storage conditions of the serum
- Do not use the serum after its expiry date
- Store serum in an area protected from light
- Manipulate serum in aseptic conditions (e.g.: under laminar air flow)
- Wear clothes adapted to the manipulation of serum to avoid contamination (e.g.: gloves, mask, hygiene cap, overall…)
- In order to preserve all serum qualities, it is recommended to thaw out the flask, to aliquote, then to re-freeze the produced flasks rather than to thaw out and re-freeze the flask at each use.
- It is recommended to use the serum immediately after its thaw out. However, if it is not useful, it is possible to store thaw out serum, at +2°C / +8°C, until 26 weeks without significant decrease of its performances in cell culture.

The product is intended to be used in vitro for research or further manufacturing only and not for use as an Active Pharmaceutical Ingredient or food or animal feed.

Remarks:
The raw serum may be treated (Heat Inactivated, Gamma Irradiated, pH modified) before filtration for different reasons:
- Importation regulation
- Exportation necessity
- Technical or quality aspects.

To be informed if your batch is concerned by treatment before filtration, please contact Biowest.