Ref : FT.SxxxSan
Version date : 23/06/2020

Fetal Bovine Serum, Embryonic Stem Cells tested

Collected from the source :
When researchers choose their serum an important factor to be considered is the source of origin which also relates to 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.
Serox Fetal Bovine Serum is derived from clotted whole blood aseptically collected from fetus via cardiac puncture.
The serum is collected or imported and treated in agreement with the European regulations.

Filtration :
Final Filter Size: 0.1µm, x 3

Sterility :
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 Tested :
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 for the absence of the indicated viruses by inoculation to permissive cells. The revelation is made by immunofluorescence for pestiviruses. Cytopathogenic agents and hemadsorbing agents are detected by microscopic observations.

Endotoxin :
All sera are tested to determine the levels of endotoxins. Serox 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.

Haemoglobin :
The haemoglobin level is measured by spectrophotometer.

Osmolality :
Is determined by a lowered freezing temperature. The osmometer is calibrated against standard solutions.

Cell Culture :
Biological performance is assessed using a 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.

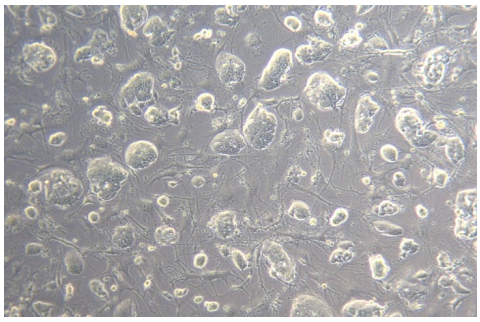
Embryonic Stem Cells tests :
Serox delegates this test to an external laboratory.

Each batch of sera is pre-screened for:
- Plating Efficiency and Proliferation,
- Morphology,
- Pluripotency markers by FACS analysis (SSEA-1, SOX2, OCT-3/4 and alkaline phosphatase (AP))

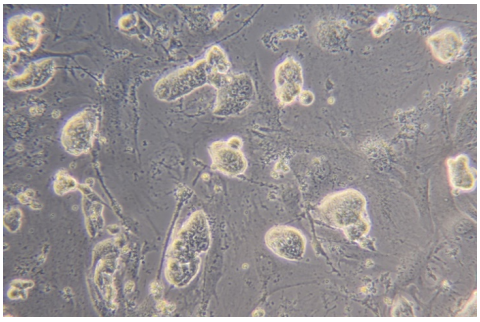
The results are compared to a control lot of serum. The biological performance is assessed using cell culture medium supplemented with a final concentration of 12% serum.

Embryonic Stem Cells Proliferation
The proliferation of the mouse ES cells was estimated on 7 days of culture with two different FBS

Embryonic Stem Cells Morphology
Mouse Embryonic Stem cells exhibit classical mouse Embryonic Stem morphological characteristics: little refractive cells, grouped in high density colonies and with well-defined walls.
During the different passages, no modification of the morphology should be observed (fig). In particular, we do not have to observe differentiation of the mouse ES cells from the first day to the last.



FBS Control



FBS Serox

Pluripotency markers
The external laboratory analyses the differentiation status of mouse Embryonic Stem cells by evaluating Embryonic Stem cell marker expression of 4 pluripotency markers: SSEA-1, SOX2, OCT-3/4 and alkaline phosphatase (AP), by Fluorescence-activated cell sorting (FACS) analysis.
FBS tested should be as efficient as FBS control in maintaining SSEA-1, SOX2, OCT-3/4 and AP expression (Ratio ≥ 0,9).

Country of Origin :
The country in which the serum was taken from the donor/animal.
BioWest sera are sourced from the following countries :

Mexico	EU approved
South America	France

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

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, in laboratory only. Do not use it in therapy, human or veterinary applications.

Note:
The raw serum may be gamma irradiated before filtration for different reasons:
- Importation regulation
- Exportation necessity
- Technical or quality aspects.

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