

ALTERNATIVES TO THE USE OF FOETAL BOVINE SERUM AND OTHER ANIMAL-DERIVED COMPONENTS IN CELL CULTURE APPLICATIONS

Foetal Bovine Serum

Each year, 600,000 litres of foetal bovine serum (FBS) are collected from up to 1.8 million foetal calves worldwide for laboratory use.¹ FBS is used as a supplement for cell culture media, providing an undefined mixture of macromolecules that maintain cell viability and facilitate cell growth in culture.²⁻⁴

Limitations of the use of FBS

Scientific Concerns

- Batch-to-batch variability: An estimated 1,800 proteins and more than 4,000 metabolites are present,⁵ which can impact reproducibility of cell-based assays and data quality.⁶
- Compromised human relevance: FBS in media may induce phenotypic changes in cell cultures, cause assay interference, and carry pathogenic elements, potentially complicating the analysis of obtained data and reduce human relevance.
- Contamination: for example, contamination of biological products with animal proteins, pathogens, and exogenous agents (e.g., endotoxins, mycoplasma, and viruses).⁷⁻⁹

Animal Welfare and Legal Concerns

- FBS is commonly collected via a large-gauge needle from the beating heart of the foetus when a pregnant cow is slaughtered. Unborn calves, who may be in the final trimester of development, are not anaesthetised at the time of blood collection and may experience severe pain.¹⁰
- A European Food Safety Authority Scientific Opinion suggests that the collection of foetal blood in this way is not permitted in the EU under Regulation (EC) No 1099/2009 and that authorisation of such a procedure is legally questionable according to Directive 2010/63/EU.^{11,12}

Alternatives to the use of FBS

Serum-free medium or human platelet lysates can replace FBS in cell culture medium. To ensure fully defined culture conditions, the goal should be to use chemically defined serum-free medium and to avoid the use of any animal-derived supplements. Recombinant proteins have the benefit of being analogous to proteins from specific species.¹³

Regulatory applications require test methods to be robust, reproducible, and human relevant. Serum-free media offer a controlled cell growth environment, improves reproducibility, and increases human relevance, thanks to the use of human recombinant growth factors and hormones.

Implementing alternatives to FBS

- Regulatory applications encourage the use of serum-free and chemically defined media as early as possible from test method development up to the regulatory implementation of the method.¹⁴⁻¹⁵
- For non-regulatory applications, many serum-free and chemically defined media options are available, along with practical guidance.^{16,17} See the Fetal Calf Serum-Free Database (fcs-free.org) for available products.

For more information, please visit www.ThePSCI.eu/FBS.

Other Cell Culture Components

Other commonly used animal-derived cell culture components with undefined compositions and batch-to-batch variability—and that can be replaced with chemically defined components or human-origin alternatives—include, but are not limited to:

- Bovine pituitary extract (BPE), which is an undefined mixture of growth factors. Depending on the application, BPE can be eliminated or replaced by supplementation with other proteins or co-culturing cell types.¹⁸
- S9 fraction, which induces metabolic activity in cultured cells. Historically derived from the livers of rats, S9 fraction can now be obtained from human livers and cell cultures and is sold by companies including, but not limited to, Scinora, ThermoFisher, Sigma-Aldrich, and BioIVT.
- Dissociation reagents derived from animals, such as trypsin and Accutase, which are often used to detach adherent cells from culture plasticware. Non-animal options, such as TrypLE, TrypZean, STEMCELL Technologies Animal Component-Free Cell Dissociation Kit, and CellPrime rTrypsin, are sold by numerous companies.
- Extracellular matrix (ECM) products, such as Matrigel, which is made by inducing tumours in mice. ECM can be replaced with non-animal ECM, such as those in Utrecht University's Basement Membrane Extract (BME)-free Database (bme-free.sites.uu.nl).

Example Case Studies Illustrating Serum Replacement with Animal-Component-Free Media

- Nessar *et al.* Promoting ethical and reproducible cell culture: implementing animal-free alternatives to teaching in molecular and cell biology. *Front Toxicol.* 2025;7:1670513.
- Rafnsdóttir *et al.* A new animal product free defined medium for 2D and 3D culturing of normal and cancer cells to study cell proliferation and migration as well as dose response to chemical treatment. *Toxicol Rep.* 2023;12(10):509-520.
- Weber *et al.* Case studies exemplifying the transition to animal component-free cell culture. *ATLA.* 2022;50(5):330-338.
- Chary *et al.* Maximizing the relevance and reproducibility of A549 cell culture using FBS-free media. *Toxicol In Vitro.* 2022;83:105423.

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