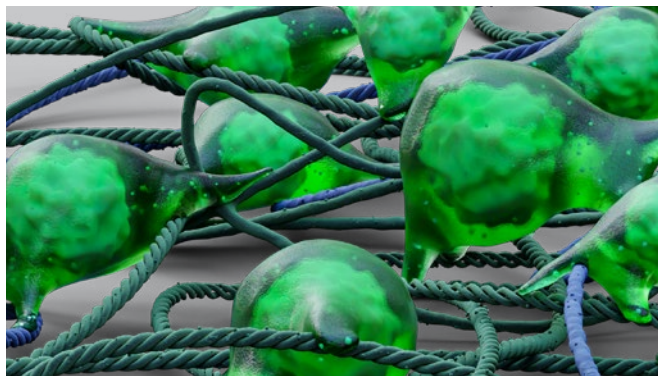


# Serum-free and Animal-free Research: More Than Just an Ethical Choice

The use of animal experimentation as a model of human physiology is a well-established practice, central to so many aspects of biomedical science, but today, more and more scientists are questioning the relevance of animal models. Critical limitations of animal subjects raise significant concerns about their reliability and predictive value for human outcomes. Furthermore, there remains longstanding ethical resistance, and mounting legislation from global governing bodies to phase out animal testing. The US Environmental Protection Agency published a directive in September 2019 which pledged that funding for animal testing will be reduced by 30% in 2025 and will be abolished completely by 2035<sup>1</sup>.

As animal testing comes under increasing scrutiny, many researchers are opting for alternative in vitro cell-based technologies to replace animal models. The cell culture market however, has long been dominated by animal-derived products, which are known to elicit unwanted experimental effects and limit reproducibility in results. This is a substantial problem for multiple sectors including biotherapeutics, where reliance upon poorly defined animal-derived culture media and reagents can be an obstacle when seeking to transition from preclinical research into clinical trials.

Many of these complications can be minimized by replacing animal/serum-containing products with highly-regulated, well-defined animal-free cell culture solutions. Animal-free and serum-free alternatives can bring a number of advantages to cell-based research, and can help to boost success when looking to scale-up production in biomanufacturing. In this article, we will consider the challenges presented by animal-derived culture media and supplements, and discover how the adoption of next generation animal-free media/supplements can improve reproducibility in cell-based research and aid biotherapeutic R&D.



## Animal-derived cell culture reagents: fear the unknown

The limitations of animal experimentation are well documented<sup>2,3</sup>, and amid growing legislative and societal pressure to move away from animal testing, many life science researchers avoid using animal subjects by instead adopting a cell-based approach to their research. Cell-based research can be tricky, and achieving reliable results is dependent upon highly standardized culture conditions which aim to preserve the structure, function, behavior, and biology of the cells in culture.

In order to maintain such delicate culture conditions, it would be advantageous to use the most precisely defined cell culture media and reagents possible. Despite this, the cell culture market is currently dominated by animal-derived cell culture media and reagents. These products are seldom well defined, and are known to come with wide batch-to-batch inconsistencies. Unbeknown to many researchers, animal-origin products can cause significant unintended experimental effects, which in turn can drive poor result reproducibility.

## The FBS conundrum

Take FBS (fetal bovine serum) for example, a universal growth supplement present in many cell and tissue culture media products. FBS is a natural cocktail comprising a multitude of factors which enable cell attachment, growth, and proliferation. Despite being widely used in cell culture for more than 50 years, FBS remains poorly characterized, but is thought to contain over 1,800 proteins and more than 4,000 metabolites<sup>4</sup>.

There are various drawbacks with FBS which can impact results in an insidious fashion. Chief among them is the lack of chemical definition and batch-to-batch inconsistencies giving rise to poor replicability in experimental results. Furthermore, FBS has immunogenic potential, and as a result, cells grown in its presence are rendered unsuitable for downstream clinical applications, such as cell therapy. FBS is also bioactive, and may contain adverse factors such as endotoxin, mycoplasma, viral contaminants, and prion proteins raising biosafety concerns.

Each of these limitations can produce wide variation in cell proliferation, differentiation, gene expression, and transcriptome stability, increasing the risk of yielding unreliable data. As a consequence, experimental results gathered using FBS-treated cells could be questioned, since any effects discovered may be a result of artifacts present in FBS, rather than the experimental variable.

## Knock-on effects in biomanufacturing and biotherapeutics

The issues drawn from animal-derived cell culture reagents are not merely confined to in vitro research. Many biomanufacturing processes rely upon animal-derived media and supplements to boost cellular growth and proliferation prior to bioprocessing. In the production of research proteins for example, if animal-derived reagents have been used to boost cellular growth prior to bioreactor inoculation, it can bring undesirable consequences, made manifest by wide batch-to-batch variability, poor yield, and variable quality, and could negatively affect the safety profile of the product produced.

Animal-derived cell culture products give rise to significant problems in the biotherapeutics/regenerative medicine sector too. Since animal-derived products carry an increased risk of contamination from animal components or byproducts, their use in therapeutic research and development can become an obstacle when seeking regulatory approval. The same goes for recombinant RUO/GMP proteins – many of the commercially available recombinant proteins used

in biotherapeutic R&D are not manufactured with an animal-free workflow, and as such, could carry traces of contaminants or pathogens.

The drawbacks presented by animal-derived cell culture raises myriad issues for those looking to scale-up production or transition from preclinical research to clinical trials. But what can be done to combat this?

## Animal-free cell culture technologies

As we have seen, there are scientific, ethical and practical arguments to be made for the adoption of animal-free cell culture. Only in more recent years though, have viable alternatives emerged onto the market. Today, a host of animal-free cell culture media and supplements have become commercially available.

This next generation of cell culture products are characterized by their high chemical definition, and many are completely free from animal-derived components. All of the chemical constituents and their concentrations are known, helping to eliminate batch-to-batch variation and improve results replicability. For biomanufacturing applications, animal-free cell culture media stands to improve product quality and yield by eliminating negative effects of animal-derived products while ensuring that the cell culture is maintained at optimum conditions during bioprocessing.

## Animal free product nomenclature

For those seeking animal-free cell culture products, there are several key descriptive phrases used:

- **Animal-free** - Animal-free products are never exposed to potential contamination by animal products at any point during their manufacturing.
- **Serum-free** – Completely free from serum (both animal and human-derived). Beware: some commercially available ‘serum-free’ media are not animal-free as they contain animal-derived supplements other than serum i.e. not optimal replacements for FBS-supplemented media as they can still cause unwanted effects.
- **Xeno-free** – Free from any animal-derived products, but may contain human-derived components, such as human serum, holo-transferrin, or insulin.
- **Chemically defined** – Completely free from serum (both animal and human-derived). All of the chemical components and concentrations are known, eliminating unwanted effects and improving quality control.

## Commercially available animal-free solutions

Now that some of the nomenclature is more clear, let's take a look at some of the animal-free cell culture products commercially available:

**Animal-free/serum-free cell culture products** – A host of animal-free cell culture media and supplements are now available. These alternatives to traditional culture media can boost results replicability, and require no supplementation with serum to ensure strong culture growth, attachment, and proliferation. Bio-Techne, have developed a range of serum-free, xeno-free culture products, including the [ExCellerate™](#) line of culture media, optimized for cell type and applications including stem cell culture, immune cell culture, and neuronal cell culture.

**Animal-free cytokines and growth factors** – We also offer a wide catalogue of cytokines and growth factors which have been carefully manufactured using a complete animal-free workflow. All of the production and purification procedures use equipment and media that are certified animal-free. This makes for reliable cytokine/growth factors that carry zero risk of contamination from animal material.

**Animal-free recombinant proteins** – It's not just cell culture products that can benefit from an animal free production workflow, we offer a comprehensive catalogue of R&D Systems™ [animal-free RUO](#) and [GMP grade recombinant proteins](#). Our RUO grade proteins manufactured in a dedicated, controlled access facility which is used exclusively for the production, purification, and bottling of Animal-Free proteins. Furthermore, they originate from the same clone, sequence, and expression system as our animal-free GMP-grade proteins, and they are manufactured using the same methods, providing for a seamless transition from preclinical research into clinical manufacturing.

## Benefits of adopting animal-free culture media/supplements

There are numerous benefits that adopting serum-free animal-free cell culture media can bring to in vitro research:

- High reproducibility** → Unparalleled consistency for replicable results
- Non-infectious** → Complete animal-free manufacturing setup eliminates risk of contamination
- Non-immunogenic** → Streamline your transition from RUO to GMP
- Custom options** → Unique media compositions available for specialist cell culture applications
- Smooth supply chains** → Ethical and reliable supply chains dedicated to animal free research

## Advantages in biotherapeutics and biomanufacturing

With animal-free cell culture solutions and RUO/GMP proteins now commercially available, stakeholders in the biotherapeutics and biomanufacturing sectors have a viable alternative that can eliminate concerns of contamination, and provide stability and reliability to R&D and manufacturing processes. For those seeking to scale-up production or push their research from preclinical studies to clinical trials, starting from assured animal-free media, reagents and recombinant proteins can provide the best chances of success, and provide peace of mind with improved safety profiles and simplified regulatory pathways.

## Why not go serum-free, animal-free today?

The evidence is clear. The use of animal-derived cell culture products and recombinant proteins are not only problematic from an ethical point of view, but also bring significant limitations to experimental results. Now that viable alternatives are becoming commercially available, researchers should consider adopting animal-free solutions into their research and benefit from strengthened, more reliable results, as well as a clear conscience.

## Get help from the experts

Our Cell Culture Media technical Services team at R&D Systems can provide recommendations on switching your cell culture to serum-free, animal-free alternatives for your specific cell types and applications. Learn more: <https://www.bio-techne.com/reagents/cell-culture-reagents/serum-free-animal-free>.

## Can't find what you're looking for?

Contact us at: [info@bio-techne.com](mailto:info@bio-techne.com) for more info, or at: [sales.emea@bio-techne.com](mailto:sales.emea@bio-techne.com) for advice from our sales team.

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