BOCASCIENTIFIC

We Bring The World Of Biotechnology To You

Animal-Free Growth Media Solutions for Cellular Agriculture

bocascientific.com

Boca Scientific introduces Multus' animal-free, serum-free products

Cultivated meat is an artificial meat that eliminates the need to raise and farm animals for food. Culturing cells using animal-free raw materials ensures batch-to-batch consistency for reproducible cultivated meat production. Multus provides animal-free, serum-free growth supplements to replace the commonly employed fetal bovine serum (FBS) growth supplement, which is prone to compositional variability. Multus also provides animal-free attachment factors for ECM scaffolds. Alone or in combination, these effective solutions promote cell proliferation in different cell types, making them ideal for small-scale and industrial production of cultivated meat.

Meat and poultry are important sources of protein, vitamins and minerals for diets. Since the 1980s, the global demand for livestock-derived food has continued to increase due to changing diets, rising incomes and increased population growth. Meat production and demand are forecasted to double by 2050[1] compared to 2012. Livestock is a major source of income for developing and developed countries[1], supporting the livelihoods of 1.3 billion people worldwide[2]. The disadvantage of intensive farming is its detrimental effect on the environment: livestock feed is the largest user of land resources and supplies[3] and is one of the main contributors to climate change[4,5]. One solution for reducing meat consumption is using more meat alternatives, which have environmental benefits.

Meat alternatives: cultivated meat

Cultivated meat or cultured meat is a type of animal meat produced by cultivating animal cells, which serves as an alternative to beef and poultry. The cultivated meat industry is growing fast – estimated to be worth $275.59 million in 2050 at a compound annual growth rate (CAGR) of 21.2%[6]. Cultivated meat has a low carbon footprint, and only a fraction of the natural resources (water and land usage) are needed compared to conventional meat production. In addition, only a few animals are required to source cells for cultivated meat. Using fewer animals would, reduce both food-borne illnesses and the use of antibiotics in animals thus minimizing the spread of antibiotic resistance[7].

Cell sources for cultivated meat

Sourcing cells is the first stage of the workflow for cultivated meat production. Cultivated meat relies on the initial harvesting of cells from animals. Primary adult stem cells are one option, obtained from tissue biopsies or post-mortem tissues. Pluripotent cell sources – either embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) – are another option. Primary cells can also be isolated from animals. With adult stem cells, skeletal muscle tissues collected from the animal can be used to isolate muscle-resident progenitor cells. As embryonic stem cells are pluripotent, they can give rise to any non-reproductive cell type. iPSCs need to be reprogrammed back into an embryonic-like pluripotent state by differentiating into the mesodermal cell lineage to obtain muscle-resident progenitor cell types[8]. Stem cells can differentiate into the cell types that make meat, including myoblasts/myocytes, adipocytes (fat cells), and fibroblasts[9]. Once a sufficient number of cells are obtained, with reports stating 10^{12}–10^{13} cells needed for 10–100 kg of meat[10], the progenitor cells can be terminally differentiated into mature cells or tissues.

One challenge of producing cultivated meat revolves around either growing the sourced cells or the issues with the type of cells obtained. Obtaining quality cell lines is a major consideration. The Good Food Institute explains that, ideally, multiple cell lines could be generated from individual animals and different biopsy locations within the animals. Cultivated meat from cell lines can also be influenced by factors such as age, sex, species, or breed. Developing cell lines or stem cells for cultivated meat is a time-consuming process, and there is a lack of standardization in characterization methods, which can affect the maintenance of quality products.

Batch-to-batch variability of conventional growth supplements

Essential raw materials for growing cultivated meat are growth media and media supplements. Indeed, these costs contribute to between 55–95% of the final cost of the product[11]. Cultivated cells are grown in a growth media with nutrients. More specifically, a pH-balanced growth media contains amino acids, vitamins, inorganic salts, and a source of energy — glucose. Serum is added to the growth media to serve as a source of growth factors, along with hormones, lipids, amino acids, and minerals. These support cell proliferation, cell maintenance, cell adhesion, and cell signaling; all essential processes in growing cultured cells. Serum also helps to protect cells against mechanical damage in stirred cultures. This is particularly important when growing cultures in stirred tank bioreactor vessels.

Expanding cells is the first step of producing cultivated meat. Growing cells for cultivated meat, first, involves growing cells in standard cell culture flasks with growth media containing serum. The serum is used at a final concentration of between 5-20% of media, depending on the cell type. Large volumes of growth-supplemented media are used to grow cultured cells in bulk quantities. Every few days, old media may need to be removed to be replaced with fresh, supplemented media. This is needed to replenish nutrients, keep the correct pH, and eliminate waste products and extracellular signals produced by the cells.

A greater volume of supplemented media is needed as the number of cells increases. Growing the quantities of cells at the scale required for producing cultivated meat then requires

transferring the actively growing and dividing culture cells into a bioreactor. Large volumes of growth media are needed for bioreactors — more than several thousands of litres[12].

There are several problems with using FBS as a growth supplement. Firstly, FBS is animal-derived, and so does not fit with the ethos of avoiding the use of animals. Also, the FBS market is highly volatile, with price fluctuations stemming from the availability of raw materials. To compound this problem, the price of FBS is becoming increasingly more costly. This variable pricing places a strain on laboratory and production budgets. The availability and increasing cost of FBS are caused by multiple factors, including variations in livestock numbers, livestock feed prices, and weather conditions[13].

Another problem with FBS is the batch-to-batch variation, which arises from using animals from different origins, and manufacturing facilities, the fact that collection and processing procedures may be different, as well as the differing collection dates. The batch-to-batch variability of FBS deriving from different lots and sources leads to variability in the biochemical composition of FBS. Although governments work alongside the World Organisation for Animal Health (founded as Office International des Epizooties) to regulate the collection and manufacture of serum, many companies lack documentation to support the health of animals, contamination risk, and biochemical profile of the sera. This variation in biochemical composition, in turn, leads to variations in cell growth rates and other cellular processes. As FBS is not included in food, employing FBS has additional food safety considerations.

Boca Scientific's new supplier, Multus, provides an animal-free alternative to FBS

Boca Scientific's new supplier, Multus, is addressing the variability and availability of FBS by providing an animal-free serum- free alternative. Multus is a UK-based company founded by scientists. Initially a startup from Imperial College London, the company is now based in White City Innovation District, London, and produces an efficient and reliable animal-free serum-free alternative as well as protein products to support cell culture.

Multus is committed to producing high-quality cellular agriculture growth media and associated ingredients. Their recent ISO 22000 accreditation means that their products can be in the food supply chain with the reassurance that safe food will be produced. ISO 22000 is the international standard for food safety management systems. It is based on the Hazard Analysis and Critical Control Point system (HACCP), a framework for analyzing and controlling biological, chemical, and physical hazards from procurement of raw ingredients to manufacturing and distribution. ISO 22000 accreditation further validates Multus' commitment to identifying and managing potential food safety hazards at all steps of their production and testing validation procedures.

Proliferum® LSR from Multus

Proliferum® LSR from Multus is a serum-free alternative to FBS in growth media, which can support the optimal proliferation of different mammalian cell lines, including myoblasts, fibroblasts and adipocytes (Figure 1). The animal- and serum-free alternative to FBS is suitable for adherent cells, suspension cells, cell isolation, cryopreservation and recovery. Multus rigorously tests their reagents against FBS for their cell proliferation rates. Multus' Proliferum® LSR produces higher proliferation rates over extended time periods and multiple passages than FBS (Figure 1).

Multus' Proliferum® LSR can be used at the same concentration as FBS for a range of cell applications, from adherent cell culture to suspension culture. Employing Proliferum® LSR, unlike FBS, delivers consistent batch-to-batch in cellular processes with reproducible cultivation of cells.

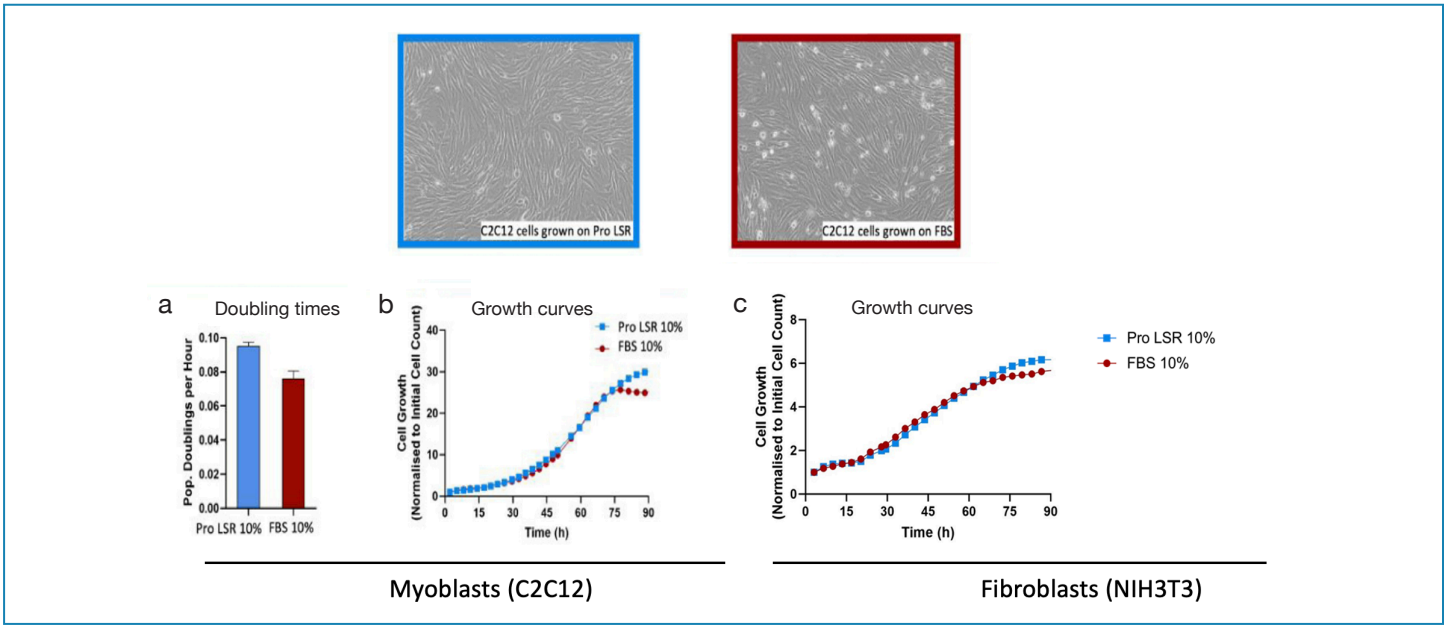


Figure 1. Multus' Proliferum® LSR produces higher proliferation rates of C2C12 myoblast cells and NIH3T3 fibroblasts over extended periods Growth media (DMEM/F12) supplemented with Proliferum® LSR at the same concentration as FBS produces higher doubling times (a) and cell growth over sustained periods (b and c).

Multus' animal-free FBS alternative, Proliferum® LSR

Key features

- Growth supplement for well-developed expansion of primary and immortalized cells
- Suitable for small-scale and industrial production (using bioreactors)
- Assured batch-to-batch consistency ensuring reproducibility in cell proliferation rates
- Animal-origin free (does not contain animal-derived materials)
- Works effectively with different scaffolds (collagen, gelatin, Geltrex™)

Characteristics

- Free of bacteria and fungi, determined by sterility testing using molecular detection
- Mycoplasma-free ensured by highly sensitive and specific testing
- Negative for the presence of particulate/contaminates
- Endotoxin < 10 EU/mL
- Functional testing (cell proliferation: cell doubling time and cell growth over several days) shows efficient proliferation
- A certificate of analysis is available for each lot of products detailing the results of the referenced testing

*Currently this product is for R&D use only.

Product	Size (catalog number)
Proliferum LSR	10ml (Cat# PLSR01)
	100ml (Cat# PLSR100)

Contact us for pricing for larger volumes

Animal-free ECM proteins

Cultivated meats should be structured and thick to successfully mimic meat. In living organisms, the extracellular matrix (ECM) – the three-dimensional network of macromolecules secreted or produced by cells – provides structure to cells and tissues. The structural support imparted by the ECM are integral to forming local adhesion complexes that affect cell signaling and influence cellular processes, such as cell attachment, cell polarity, migration, and differentiation.

The last production step for cultivated meat relies on the use of a scaffold that supports cell development and provides cultivated meat with a three-dimensional structure and texture. These scaffolds use attachment factors resembling extracellular matrix (ECM) interactions. The attachment factors are essential in maintaining cells in culture after cells are passaged from one culture flask to another – for example, when cells have reached confluence (when insufficient room remains for cells to grow as a monolayer in a culture vessel) and need to be transferred into new culture flasks. In this case, cells are detached from their previous support using enzymes such as trypsin.

Cells need specific attachment factors such as vitronectin to adhere to the tissue culture plastic or in bioreactors. This is because several cell types are anchorage-dependent and need to adhere to a scaffold surface before they can proliferate and differentiate properly. Vitronectin is also very important for the adhesion of stem cells, such as bovine muscle stem cells[14]. Vitronectin avidly binds glycosaminoglycans (GAGs)[15], which are complex carbohydrates naturally expressed ubiquitously and abundantly on the cell surface and in the extracellular matrix. GAGs include heparan sulfate, chondroitin sulfate, dermatan sulfate, and keratan sulfate. GAGs are essential as scaffold proteins in cultured meat as they have a myriad of roles in cell signaling processing, including regulation of cell growth, proliferation, and promotion of cell adhesion. Multus' rh-Vitronectin is a

Vitronectin

truncated recombinant form of the wildtype human Vitronectin (VTN-N) fused with a 5-histidine tag. The vitronectin glycoprotein is produced to a high purity (Figure 2). Other scaffolds used in cultivated meat, such as collagen and gelatin, are predominately animal-derived[16]. Employing the highly recombinant purified rh-Vitronectin from Multus eliminates variability from the scaffold for cultivated cells.

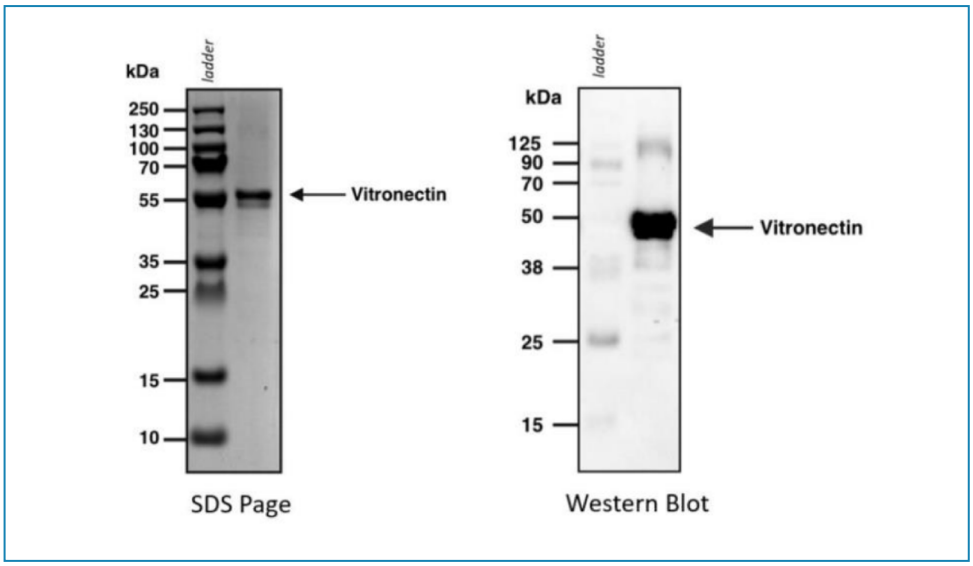


Figure 2. SDS-PAGE and western blot analysis of purified rh-Vitronectin from Multus

Multus' routine validation testing uses different cell types to compare rh-Vitronectin (Multus) against other commercial coatings in cell attachment and cell proliferation assays. Cell adhesion is either higher with Multus' rh-Vitronectin than commercial vitronectin in C2C12 cells or comparable in NIH3T3 (Figure 3a). In addition, Multus' rh-Vitronectin has little variability in the attachment rates (Figure 3a). Rh-Vitronectin performs consistently well and achieves sustained cell growth (Figure 3b).

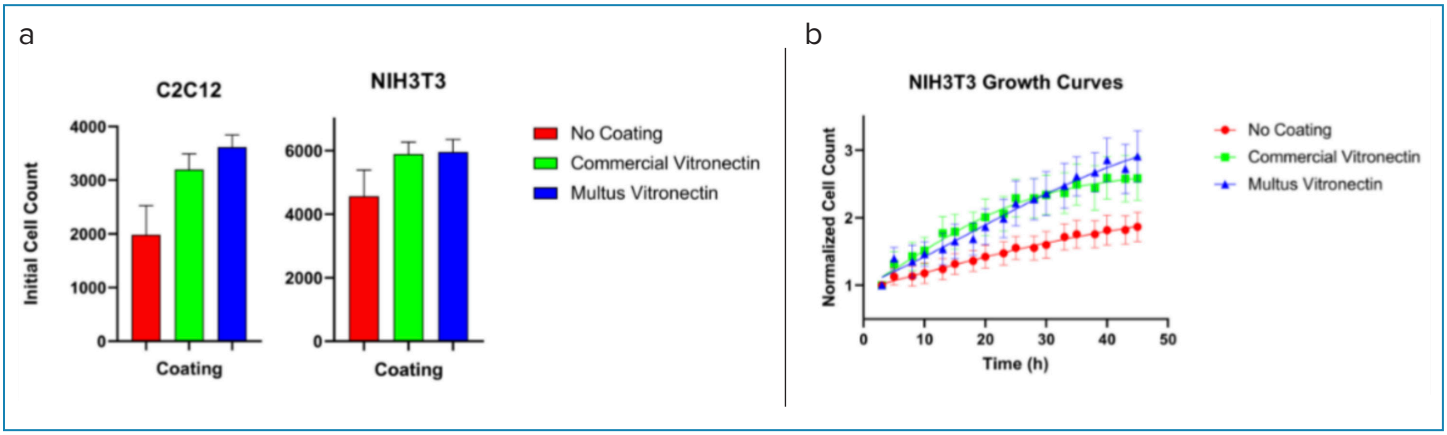
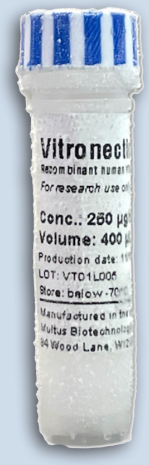


Figure 3. Routine validation testing of rh-Vitronectin from Multus using cell adhesion (a) and proliferation assays (b) rh-Vitronectin (Multus) tested against no coating or a commercially available vitronectin coating.

Multus' Recombinant human rh-Vitronectin

Key features



- **Recombinant human Vitronectin can provide anchorage to promote cell proliferation and supports normal colony morphology for different cell types**
- **Food safe and ISO 22000 certified**
- **Animal-free ECM protein — does not contain animal material**
- **Usage defined for small- and large-scale production**

Characteristics

- **Free of bacteria and fungi, determined by sterility testing using molecular detection**
- **Mycoplasma-free ensured by highly sensitive and specific testing**
- **Negative for the presence of particulate/contaminates**
- **Endotoxin < 10 EU/mL**
- **Functional testing with cell proliferation assays demonstrates rh-Vitronectin performs effectively as an ECM protein**

*Produced under ISO22000 food safe manufacturing standard. Multus is working towards establishing a complete safety profile for using this product in food production. The use of this item is subject to Multus Biotechnology Limited's Terms and Conditions of Use.

Product	Size (catalog number)
rh-Victronectin	.1mg (Cat# VT01)
	.1mg 5-pack (Cat# VT01-5)

Contact us **for pricing for larger volumes**

Boca Scientific's reliable and efficient FBS-replacement growth supplements and animal-free ECM proteins

Boca Scientific introduces Proliferum® LSR from Multus, an animal- and serum-free growth supplement. Proliferum® LSR delivers consistent and reproducible results for producing cultivated meat, circumventing the issues faced with FBS, including batch-to-batch variability, inconsistent availability, and price volatility. Multus' recombinant human rh-Vitronectin provides an effective animal-free scaffold supporting cell proliferation in cultivated meat. These solutions are suited for small and large-scale production of cultivated meat.

Please get in touch with us if you need help and further information about any of our products.

References

1. Food and Agricultural Organization of the United Nations, The future of food and agriculture: Alternative pathways to 2050, Rome, 2018. https://www.fao.org/3/CA1553EN/ca1553en.pdf.

2. Food and Agriculture Organization of the United Nations, Animal Production, (2023). https://www.fao.org/animal-production/en/.

3. S. Manceron, T. Ben-Ari, P. Dumas, Feeding proteins to livestock: Global land use and food vs. feed competition, OCL - Oilseeds and Fats. 21 (2014) D408. https://doi.org/10.1051/ocl/2014020.

4. J. Bellarby, R. Tirado, A. Leip, F. Weiss, J.P. Lesschen, P. Smith, Livestock greenhouse gas emissions and mitigation potential in Europe, Glob Chang Biol. 19 (2013). https://doi.org/10.1111/j.1365-2486.2012.02786.x.

5. P.J. Gerber, Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO), Rome. 2013.

6. Businesswire, Cultured Meat Global Market Trends and Growth Forecasts: A $275.59 Million Market by 2025 - ResearchAndMarkets.com, (2021). https://www.businesswire.com/news/home/20211230005088/en/Cultured-Meat-Global-Market-Trends-and-Growth-Forecasts-A-275.59-Million-Market-by-2025---ResearchAndMarkets.com.

7. T.P. Van Boeckel, E.E. Glennon, D. Chen, M. Gilbert, T.P. Robinson, B.T. Grenfell, S.A. Levin, S. Bonhoeffer, R. Laxminarayan, Reducing antimicrobial use in food animals, Science (1979). 357 (2017) 1350–1352. https://doi.org/10.1126/science.aao1495.

8. J. Reiss, S. Robertson, M. Suzuki, Cell sources for cultivated meat: Applications and considerations throughout the production workflow, Int J Mol Sci. 22 (2021). https://doi.org/10.3390/ijms22147513.

9. Good Food Institute, Deep dive: Cultivated meat cell lines, (2023). https://gfi.org/science/the-science-of-cultivated-meat/deep-dive-cultivated-meat-cell-lines/.

10. V. Bodiou, P. Moutsatsou, M.J. Post, Microcarriers for Upscaling Cultured Meat Production, Front Nutr. 7 (2020). https://doi.org/10.3389/fnut.2020.00010.

11. S. Hubalek, M.J. Post, P. Moutsatsou, Towards resource-efficient and cost-efficient cultured meat, Curr Opin Food Sci. 47 (2022). https://doi.org/10.1016/j.cofs.2022.100885.

12. Good Food Institute, Deep dive: Cultivated meat bioprocess design, (2023). https://gfi.org/science/the-science-of-cultivated-meat/deep-dive-cultivated-meat-bioprocess-design/.

13. D.W. Jayme, D.A. Epstein, D.R. Conrad, Fetal bovine serum alternatives, Nature. 334 (1988). https://doi.org/10.1038/334547a0.

14. A.J. Stout, A.B. Mirliani, M.L. Rittenberg, M. Shub, E.C. White, J.S.K. Yuen, D.L. Kaplan, Simple and effective serum-free medium for sustained expansion of bovine satellite cells for cell cultured meat, Commun Biol. 5 (2022) 466. https://doi.org/10.1038/s42003-022-03423-8.

15. B.R. Tomasini, D.F. Mosher, Vitronectin, Prog Hemost Thromb. 10 (1991) 269–305. https://pubmed.ncbi.nlm.nih.gov/10608816/.

16. J.S.H. Seah, S. Singh, L.P. Tan, D. Choudhury, Scaffolds for the manufacture of cultured meat, Crit Rev Biotechnol. 42 (2022) 311–323. https://doi.org/10.1080/07388551.2021.1931803.

BOCASCIENTIFIC
We Bring The World Of Biotechnology To You

Boca Scientific Inc.

990 Washington St.
Suite 105
Dedham, MA 02026 USA

Phone: 781-686-1631
Fax: 561-995-5018
Toll Free:866-227-6335

E-mail: info@bocascientific.com
Technical Support: tech@bocascientific.com
Orders: sales@bocascientific.com