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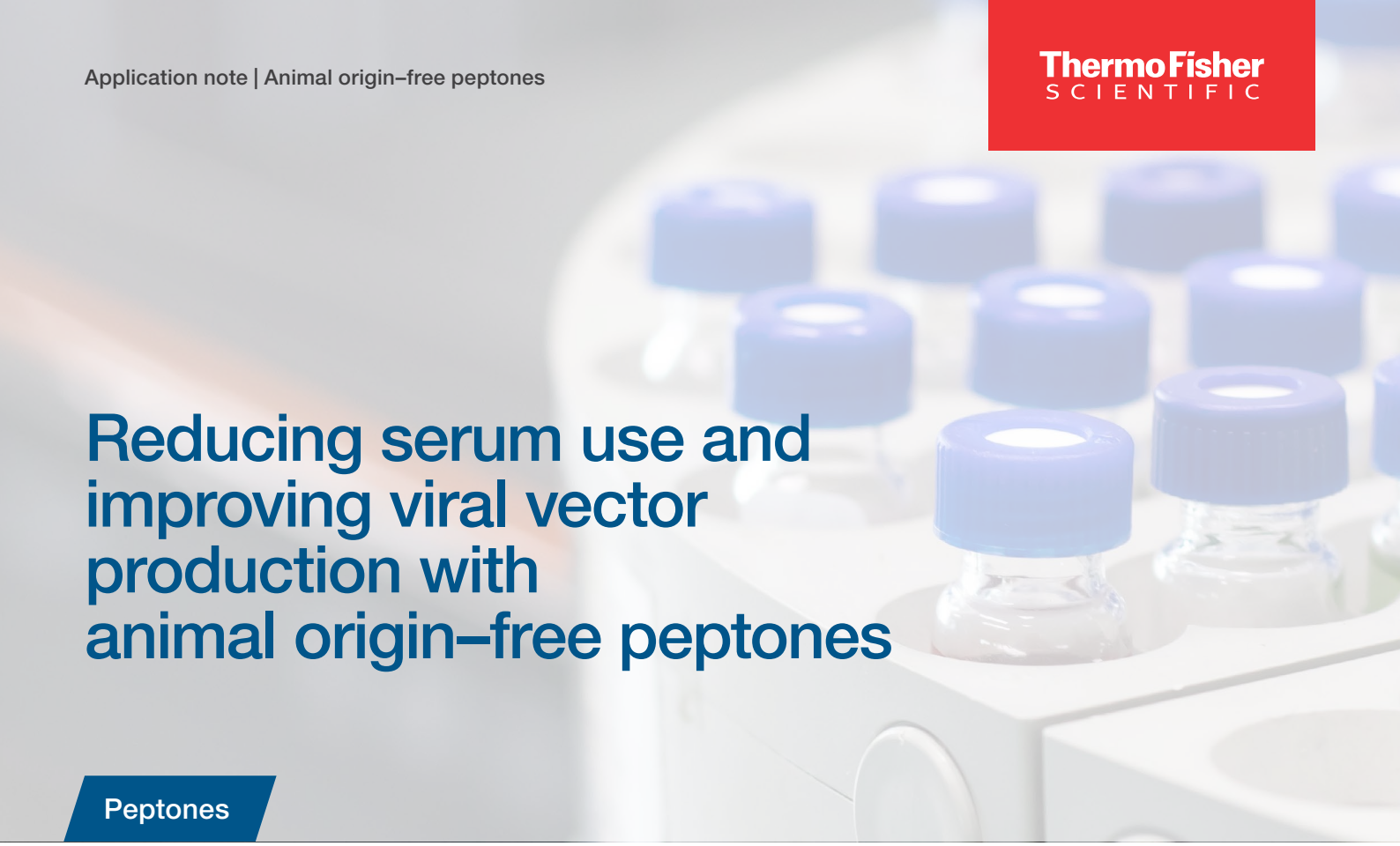

Reducing serum use and improving viral vector production with animal origin–free peptones

Peptones

Introduction

To improve the reliability, consistency, and safety of their products, vaccine manufacturers are gradually shifting away from using animal serum in their processes. Supplementing cell culture media with animal origin–free (AOF) peptones can be an economical and efficient alternative to using serum in fed-batch and reduced-serum processes. Peptones, or protein hydrolysates, consist of peptide fragments and other nutritional components derived from the hydrolysis of plant, yeast, or animal tissue. Using AOF peptones can alleviate the concerns of stakeholders who want to move away from animal-derived components. Incorporating the right peptone or peptone blend into a serum-free or reduced-serum process can also increase viral vector yield and potentially reduce the cost of vaccine manufacturing.

In two recent case studies, scientists at Thermo Fisher Scientific set out to demonstrate the value of AOF Gibco™ peptones (Table 1) for reducing serum usage and improving viral vector yield. In the first case study, the researchers evaluated the impact of AOF peptones on the growth of adherent Vero cells in reduced-serum medium and production of vesicular stomatitis virus (VSV). Five individual AOF peptones and several peptone blends with expanded nutritional profiles were tested to determine the best feeding strategy. The second case study evaluated whether AOF peptones or peptone blends could boost suspension HEK293 and BHK-21 cell growth and adenovirus production by HEK293 cells.

Table 1. Gibco™ peptones evaluated in case studies.

Product	Cat. No.
Difco™ Phytone™ Supplement, ultra-filtered	210931
Difco™ TC Yeastolate, ultra-filtered	292804
Difco™ Soytone	212488
Soy 100 peptone	670138
Cotton 100 peptone, ultra-filtered	215381
Cotton Peptone 200, ultra-filtered	215382
Bacto™ Malt Extract	218630
Wheat 100, ultra-filtered	670140

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Materials and methods

Case study 1

The Vero cell line is the most widely used adherent cell line for production of human and animal viral vaccines. Researchers evaluated the impact of five AOF peptones and several peptone blends on adherent Vero cell growth and virus production. Vero cells were first grown in Gibco™ DMEM/F-12 HEPES medium (Cat. No. 11330032) supplemented with 10% fetal bovine serum (FBS). The cells were then adapted over four passages in DMEM/F-12 HEPES medium supplemented with 2% FBS and a peptone or peptone blend at 4 g/L. The peptones included Cotton Peptone 200, Wheat 100 peptone, Difco Soytone, Difco TC Yeastolate, and Difco Phytone Supplement. DMEM/F-12 HEPES medium supplemented with 10% FBS served as the control medium. Viable cell density (VCD) and cumulative population doublings were measured throughout culturing.

To evaluate virus production under reduced-serum conditions, adapted cells were seeded in T75 flasks. When confluency reached 90–100%, the cells were washed with Dulbecco's phosphate-buffered saline (DPBS) containing Ca^{2+} and Mg^{2+} and infected with vesicular stomatitis virus at a multiplicity of infection (MOI) of 0.0001 prepared in 2 mL neat DMEM/F-12 HEPES medium. After 1 hour of virus adsorption, 18 mL of DMEM/F-12 HEPES medium supplemented with a peptone or peptone blend and 2% FBS was added to each flask. The infected cells were incubated at 37°C under 5% CO_2, and harvest was performed on day 2 post-infection. After one freeze-thaw cycle, VSV titers were determined with a plaque assay.

Case study 2

Suspension HEK293 cells (Gibco™ Viral Production Cells 2.0, Cat. No. A51218) were cultured under simple fed-batch conditions in Gibco™ Dynamis™ AGT™ Medium (Cat. No. A2617504) the workflow is described in Figure 1. Cells were seeded in triplicate at 0.7 x 10^6 cells/mL and cultured in Ambr™ 15 bioreactors (Sartorius). The test cultures were supplemented on day 0 and day 2 with an AOF peptone or peptone blend at 2 g/L or 6 g/L. The peptones included Difco Phytone Supplement, Difco TC Yeastolate, Difco Soytone, Soy 100 peptone, Cotton 100 peptone, Cotton Peptone 200, and Bacto Malt Extract. Control cultures were fed only with Gibco™ EfficientFeed™ C+ 2X Supplement (Cat. No. A3937601) at 5% on day 0 and day 2. VCDs were monitored daily for up to 6 days.

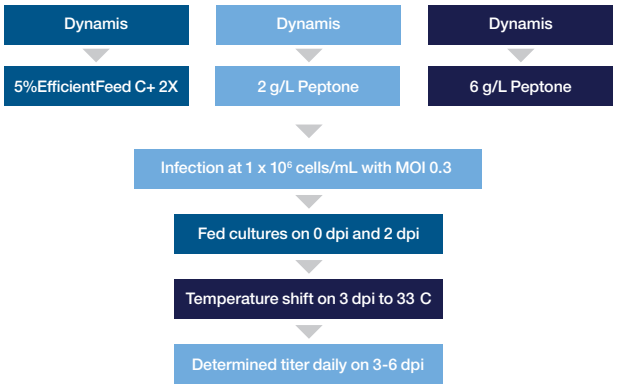

Figure 1. Experimental design for evaluation of adenovirus production in Dynamis **Medium with peptones**. Internal suspension HEK293 cells were seeded in Ambr15 microbioreactors at 1 × 10^6 cells/mL and infected at MOI 0.3. Infectious virus titers were determined with a focus-forming assay. Peptone or peptone blends (2 or 6g/L) and 5% EfficientFeed C+ Supplement (2X) were fed on 0 days post infection (dpi) and 2 dpi. Temperature shift was performed on 3 dpi to 34°C. Titer was determined daily on 3–6 dpi by focus-forming assay.

Adenovirus 5 production was evaluated with HEK293 cells. Cells were cultured in Dynamis AGT Medium until the VCD reached ~1 x 10^6 cells/mL. The cells were then infected with VR-1516™ wild type human adenovirus 5 (ATCC) at a MOI of 0.3. One hour after infection, all cultures were fed with either EfficientFeed™ C+ 2X Supplement (control) or a peptone or peptone blend at 2 g/L or 6 g/L. Cultures were fed again on day 2 post-infection. Glucose was fed to 6 g/L when levels dropped below 3 g/L. The temperature was reduced 37°C to 34°C on day 3 post-infection. After three freeze-thaw cycles, viral titers were measured with a focus-forming assay.

Results

Case study 1

Vero cell growth under reduced-serum (2% FBS) conditions with 4 g/L peptone supplementation was comparable to cell growth in the control medium supplemented with 10% FBS (Figure 2A). Notably, supplementation with 2% FBS and a Wheat 100/Difco TC Yeastolate blend or Wheat 100/Soy 100 peptone blend significantly increased the VSV titer relative to supplementation with 2% or 10% FBS alone (Figure 2B).

Case study 2

Supplementing media with Difco TC Yeastolate, Difco Phytone Supplement, or a 50/50 blend of the two increased peak VCDs in suspension HEK293 and BHK-21 cultures. Peptone supplementation increased the VCD of HEK293 cells by 10–30%, and up to a two-fold increase in VCD was observed in BHK-21 cultures. Although supplementation with individual peptones did not increase the virus titer, 75/25 and 50/50 blends of Difco TC Yeastolate and Difco Phytone Supplement supplemented at 6 g/L increased virus titers by over 50% relative to the control (Figure 3).

These studies demonstrate that AOF Gibco peptones can effectively support suspension and adherent cell growth and enhance viral vector production. Peptones can be combined and tailored to optimize processes and reduce reliance on serum with comparable or better results. AOF peptone supplementation can thus be an economical and efficient option for fed-batch or reduced-serum processes in viral vector–based vaccine manufacturing. However, it is important to carefully evaluate different peptones and peptone blends in different concentrations to optimize specific processes.

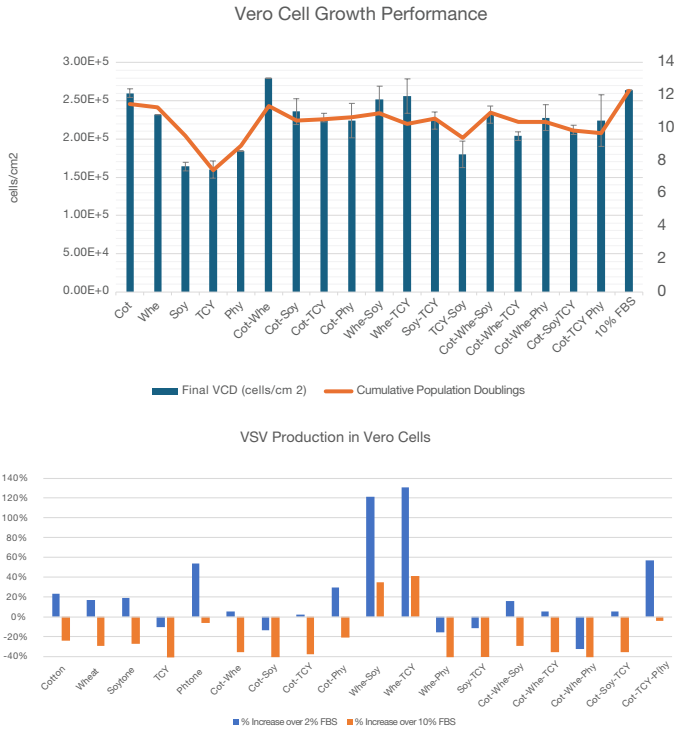

Figure 2. AOF peptones support adherent Vero cell growth in reduced-serum medium and can enhance VSV production. **(A)** Vero cell growth: peptone the cells were adapted over fourpassages in DMEM/F-12 medium with HEPES supplemented with 2% FBS and 4 g/L of a peptone or peptone blend. VCDs from the fourth passage and cumulative population doublings are shown. **(B)** VSV production by Vero cells adapted over four passages in DMEM/F-12 medium with HEPES supplemented with 2% FBS and a peptone or peptone blend. Vero cells were seeded in T75 flasks and infected with VSV at a MOI of 0.0001 once the cell density reached 90–100% confluency. On day 3 post-infection, most flasks were observed for cytopathic effect and harvested. After one freeze-thaw cycle, the virus samples were aliquoted to measure viral titers with a plaque assay.

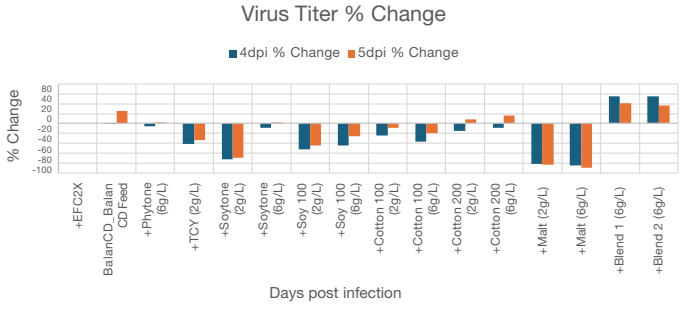

Figure 3. Human adenovirus 5 production by suspension HEK293 cells. Samples were collected on days 4 and 5 post-infection, and viral titers were quantified after three freeze-thaw cycles using a focus-forming assay. The change (%) in virus titer is shown relative to the control supplemented only with EfficientFeed C+ 2X Supplement. Blend 1: Difco Phytone Supplement (75%) + Difco TC Yeastolate (25%); Blend 2: Difco Phytone Supplement (50%) + Difco TC Yeastolate (50%).

Achieving reliable and consistent performance

Given the criticality of process consistency, it is essential to understand and control sources of variability when developing a robust bioproduction process. Variability can come from five main sources: biological factors, consumables, raw materials, process conditions, and environmental elements.

Variability can be introduced through any supplements or base media. As an example, manganese levels were assessed in 23 different lots of a peptone as well as a vitamin raw material used in a basal medium (Figure 4). The natural manganese variability among all the peptone lots was considerably less than one part per million, compared with that for the same vitamin in the basal medium, which exhibited over ten parts per million manganese content in some samples.

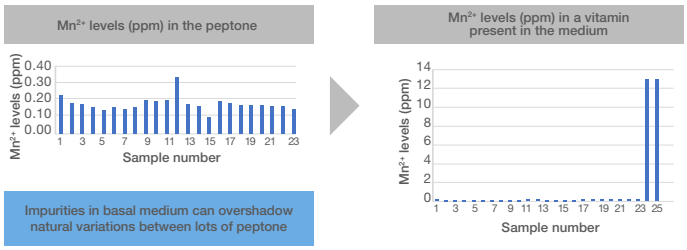

Figure 4. Manganese levels were assessed in 23 lots peptone supplement and a vitamin present in the basal medium. The manganese component levels in the peptone were all<0.4 ppm, whereas the levels in the vitamin were >12 ppm in some samples. Comparison of levels in peptone lots (1-23) and vitamin lots (24-25) are shown in the right panel.

Those results demonstrate the importance of understanding that chemically defined components are not chemically pure. Any components added to your process can introduce trace contaminants that contribute to variability. Therefore, it is important to identify and control their sources to achieve a consistent process. Working with a highly qualified bioproduction peptone supplier that offers advanced testing greatly aids in this regard.

When considering and selecting peptones in a bioproduction project, it is important to partner with a supplier that specializes in the bioproduction industry. The supplier also should offer advanced testing capabilities and knowledgeable support to help development projects achieve reliable and consistent performance. Consideration and observance of several key factors will simplify peptone screening, resulting in the rapid selection of the ideal peptones and a highly consistent bioproduction process.

We also focus on process-specific requirements, because we recognize that different peptones have varying performance characteristics depending on application. In addition to offering a wide range of peptones, we can provide analytical and key driver identification (KDI) services. Our KDI service leverages extensive technical experience with cell culture and vaccine manufacturing to identify critical peptone components that influence process performance. The KDI approach enables effective troubleshooting and problem-solving to facilitate integration of AOF peptones into manufacturing processes.

The KDI approach goes beyond the traditional test-and-hold method, in which multiple lots are evaluated with the hope of finding suitable material. We use a robust, data-driven approach that begins with analyzing the customer's data to understand process performance and identify potential key drivers. The data is then used to build a model that predicts the impact of specific analytes on process outcomes. Through iterative testing and refinement, the process narrows down potential analytes to a specific specification range that will support optimal performance for the customer's application.

By pinpointing key drivers, we can develop custom peptones with precisely defined specifications to support consistent performance and eliminate guesswork. The optimized specifications are integrated into the manufacturing process using validated analytical models to support consistent product quality with every order. For example, a customer experiencing a low lot acceptance rate (27%) was able to achieve 100% acceptance after implementing a custom peptone developed using the KDI approach.

Conclusion

Gibco AOF peptones are supported by rigorous quality control and an innovative KDI service. They can be reliable solutions for biopharmaceutical manufacturers seeking consistent and predictable process outcomes. By understanding and mitigating the inherent variability of peptones, we empower customers to optimize their processes, reduce costs, and accelerate time to market. This is especially critical in vaccine production, where consistency, scalability, and speed can directly impact public health outcomes.

Learn more at **thermofisher.com/peptones**