Using Peptones to Achieve Diverse and Demanding Bioproduction Goals

By Stacy Holdread

SPECIAL REPORT

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Gibco Difco peptones have been used for over a century to enhance diagnostic and bioproduction processes in microbial and mammalian systems. Peptones have diverse and unique nutritional profiles that make them ideal components for creating robust, high performance bioprocesses. Over the years, Advanced Bioprocessing has continued to drive innovation, design, and enhancements in cell culture media and supplements to meet evolving user needs. As the bioproduction industry grew in the 1990s, our peptones were instrumental in replacing serum to reduce risk while significantly increasing yields and overall process performance. Since that time, our peptones have been used in more than 150 biopharmaceutical products and continue to be incorporated in the development of future biotherapeutics.

Composition and Role of Peptones in Bioproduction

Peptones (hydrolysates) are water-soluble products derived from the partial hydrolysis of proteins from yeast, plants, or animal sources. A unique feature of peptones is that they bring numerous advantages to a culture with the addition of one supplement. The benefits can be grouped into three main categories: nutritional diversity, protective effects, and performance enhancement.

Nutritional Diversity: Peptone components offer a diverse and balanced composition of free amino acids, polypeptides, carbohydrates, salts, and trace metals to meet specific growth and productivity requirements of many different cell types.

Protective Effects: Peptones bring protective effects through different types of nutritional buffering, which includes protecting cells from toxic levels of media components and allows for higher concentrations of key components in the culture. Additionally, peptones have been shown to prolong culture life by delaying the onset of apoptosis.

Performance Enhancement: Peptones offer notable performance enhancements including increased cell growth and specific productivity. Additionally, peptones have demonstrated abilities to influence product quality attributes such as glycan and charge-variant profiles.

Process Considerations

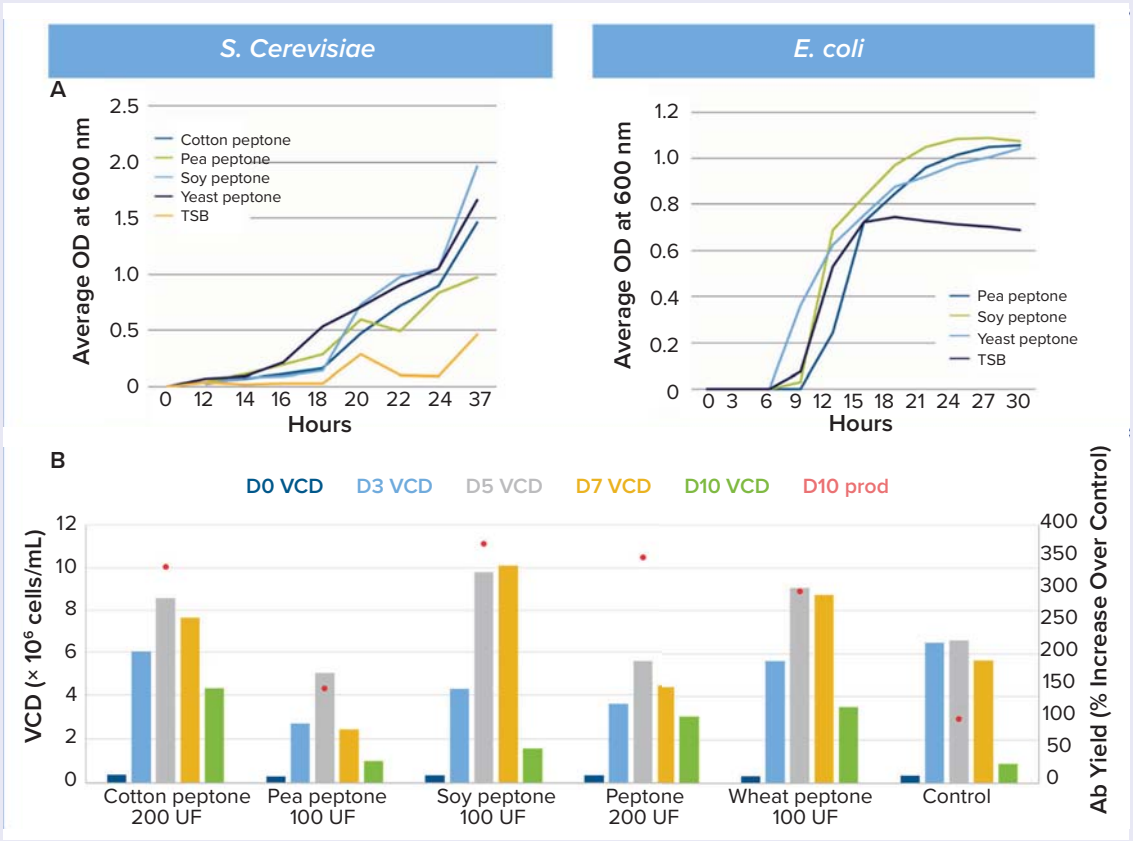
Media and supplements used in bioproduction processes typically fall into three broad categories: animal origin (AO), animal-origin–free (AOF), and chemically defined (CD). All three types can support high-performing bioproduction processes. Each one offers certain benefits and considerations that should be weighed to identify process conditions best suited to a specific project.

AO processes can contain peptones derived from animal sources including bovine, porcine, equine, and other mixed animal tissues. AO peptone processes generally are well suited for serum replacement but can prompt more regulatory considerations and inherent variation. AOF processes bring the same benefits as AO processes but with reduced risk and regulatory considerations because peptones are derived from nonanimal sources such as yeasts and plants and including soy, cotton, wheat, and pea. CD processes are designed with components produced synthetically or derived from other specifically characterized and defined sources. The advantage of a CD process is that each component and concentration is known, which reduces regulatory risks. However, CD components can be variable, so CD processes still may have inherent variability. Also, because each component must be identified and optimized, CD processes can add significant development time and cost compared with processes containing peptones. It's critical to understand the relative advantages and considerations so that whichever type of process is selected can be monitored and controlled.

Benefits of Using Peptones in Bioproduction

The unique nature of peptones makes them ideal components for bioproduction applications. The multiple benefits they bring can provide for development of robust, high-performance processes.

Figure 1: (A) Evaluation of *Saccharomyces cerevisiae* and *Escherichia coli* cultures with cotton, pea, yeast, and soy peptones compared with use of a tryptic soy broth (TSB) control medium; peptones were added to M9 salts with glucose. (B) A CHO-K1 cell line was grown in a chemically definged (CD) control medium with wheat, yeast, soy, pea, or cotton peptones added at a concentration of 5 grams per liter (OD = optical density, VCD = viable cell density)



Versatility Across Cell Types: The versatility of peptones is demonstrated by their successful use across a broad number of cell types such as yeast, bacteria, and mammalian cells. Figures 1A and 1B show how the same peptones can be used to enhance performance of *Saccharomyces cerevisiae*, *Escherichia coli,* and a Chinese hamster ovary cell line (CHO-K1). Although each peptone performed differently, they all achieved higher peak viable cell densities and produced much higher antibody yields than the CD control medium. These data demonstrate that all cell types show improved performance in the presence of peptones.

Nutritionally Rich: Each cell line and bioproduction process is different, so it's important to screen a number of peptones at different concentrations to ensure optimal conditions.

The results in Figure 2 show four peptones tested at two concentrations each in the same base medium to demonstrate how performance is influenced by these different conditions. Although all peptone-containing media supported higher performance than the CD control, overall cell performance was different for each peptone. Peptone concentration also is a factor, as shown with Peptone 2. If screening were limited to the 3 g/L condition, the higher cell density and production achieved using 9 g/L would have been missed. Figure 3 shows a similar situation with *E. coli* grown in Terrific broth, which is a highly enriched, peptone-containing medium. Through optimizing peptones along with the base medium, we developed a high-performance *E. coli* fermentation broth that provided for a significantly higher cell density.

Flexible Applications

Peptones can be used as additions to basal media and as a feed during fed-batch cultures (Figure 4). Using a peptone fed-batch process instead of a batch culture, culture life was extended by five days, from day nine to day 14. Additionally, improvement was realized in the overall antibody yield from the peptone fed-batch culture. Using a

Figure 2: Four different peptones tested at two concentrations were evaluated with a CHO cell line compared to a chemically defined (CD) control medium.

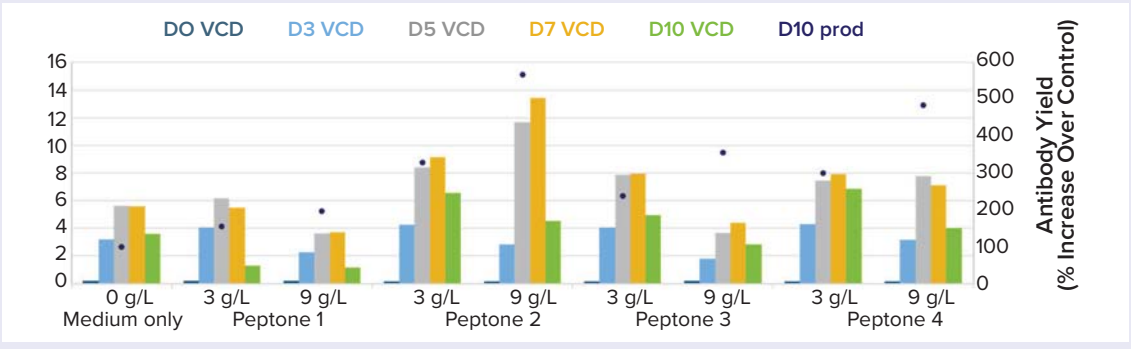
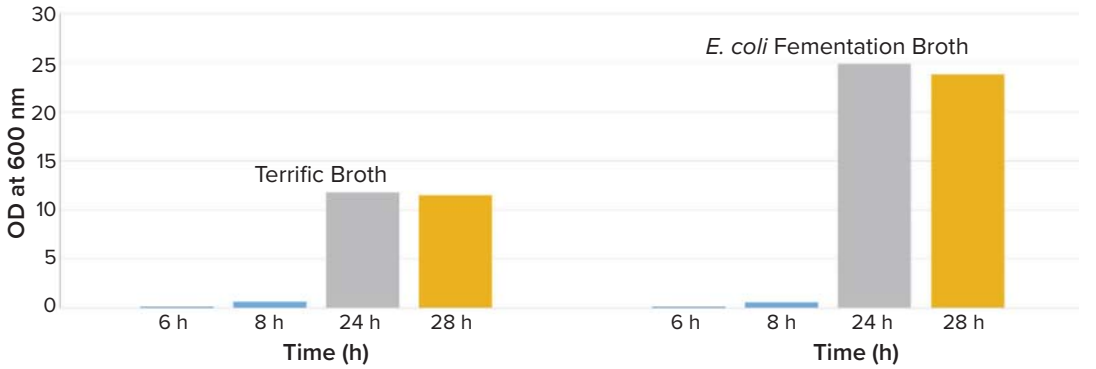


Figure 3: Growth of *E. coli* in Terrific broth compared with an optimized *E. coli* fermentation broth (OD = optical density)



peptone as a feed enabled rapid extension of culture life and increased antibody yield.

Blending peptones can enhance culture performance further. Figure 5 shows growth and production using individual and blended peptones compared with a CD control condition. The addition of individual peptones improved antibody yield while demonstrating good growth profiles. However, blending peptones showed synergistic effects that in this case were not achieved by using peptones individually. During process optimization, it can be beneficial not only to look at a number of peptones, but also to evaluate whether blending them can improve performance further.

Enabling Rapid Optimization

Peptones can enable more rapid and efficient process optimization than with development of a CD process. As reported in Figure 6, CD and peptone-containing formulations were screened to identify a formulation that could achieve a day-five viable-cell density (VCD) of at least 10 million cells/mL. In the initial screen, the peptone-containing formulation met the growth criteria whereas the best performing CD medium was well below that growth target. With nine months of additional optimization, higher growth was achieved with the CD medium, but performance still did not meet the growth target. In this example, peptone optimization was a much faster, more efficient way to successfully meet the performance target.

Achieving Target Protein Quality

Achieving desired protein quality attributes can be aided using peptones in a medium. The CD control medium reported in Figure 7 had an undesirable glycan profile that was shifted successfully to the desired profile through supplementation with a peptone or CD supplement. As Figure 8 shows, the original peptone-containing medium did not present the desired charge-variance profile. Optimizing concentrations of those peptones resulted in multiple formulations that achieved the proper profile. Although supplements can be useful tools in controlling protein quality regardless of which supplement is added, it is important to understand how each supplement will affect the product quality profiles.

Figure 4: CHO line evaluated in batch and fed batch culture (VCD = viable cell density)

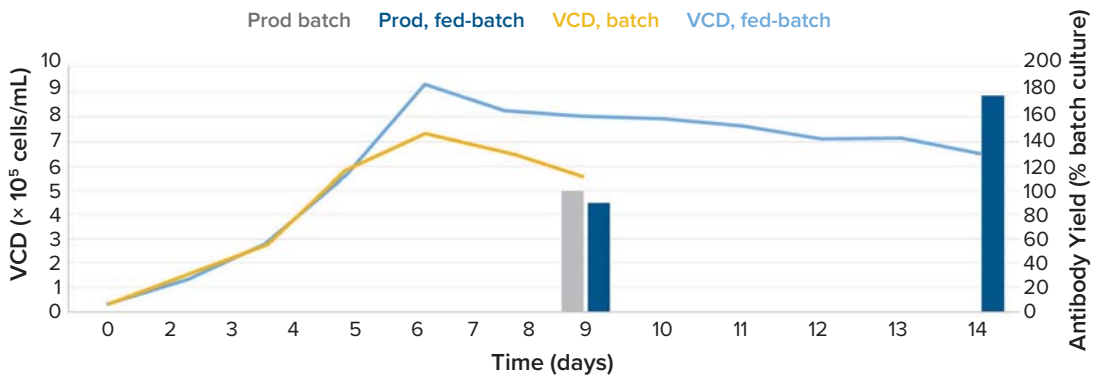
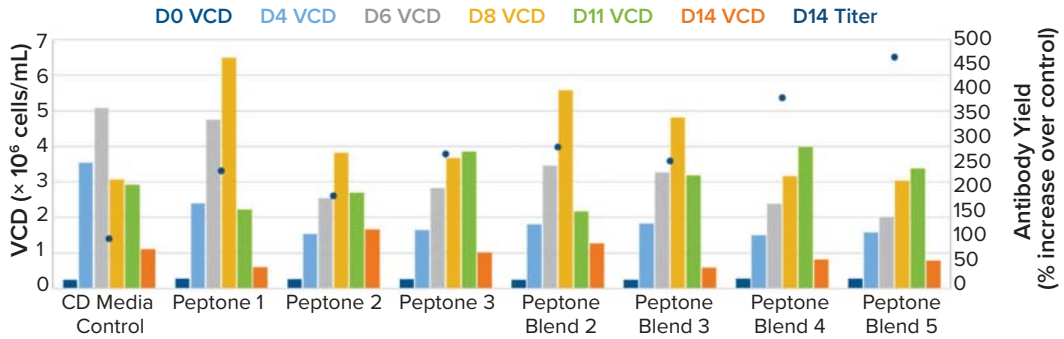


Figure 5: Performance comparison of individual and blended peptones with a CHO cell line (VCD = viable cell density)



Selecting the Right Peptone

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 9). 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.

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.

Two main considerations should be factored into peptone selection: supplier capabilities and process requirements. When selecting a peptone supplier, it is important to understand the company's industry focus, testing capabilities, and willingness and technical ability to work with a customer's process.

Because of the wide range of peptones available, it is important to focus on those that are designed and tested with bioproduction requirements in mind. In addition to traditional testing, peptones for bioproduction also should undergo specialized testing such as examination of solution clarity, endotoxin, mycoplasma, and filterability. Working with a partner that understands the use of peptones in bioproduction processes with microorganisms and mammalian cell lines increases your likelihood of developing a consistent, robust process.

Figure 6: Process optimization timeline comparison of CD- and peptone-containing media

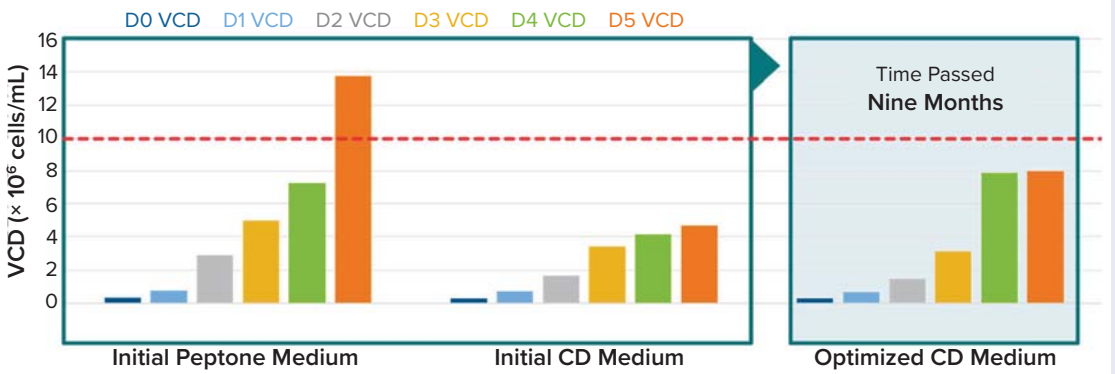
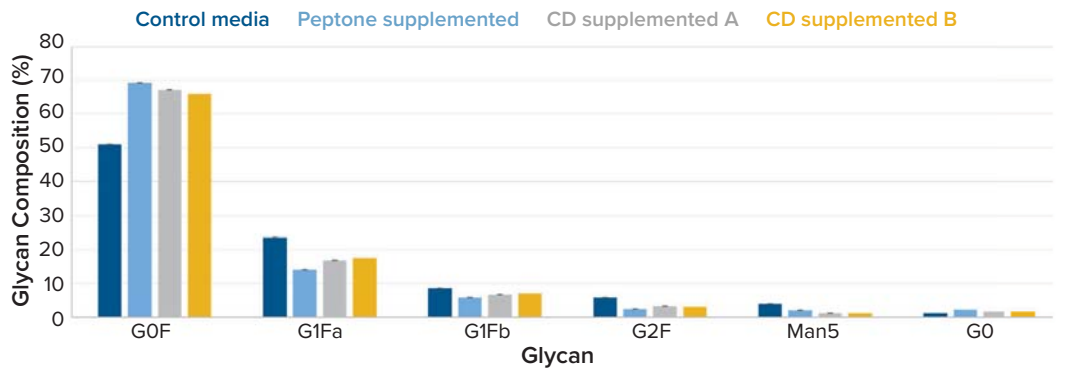


Figure 7: Achieving desired glycan profile using peptone or CD supplementation



It also is beneficial to select a supplier that offers specialized analytical capabilities and can test complex media formulations to aid in process characterization and identification of critical components. Such analytical tools can aid in understanding sources of variability and enable controls and process optimizations to maintain consistency.

Equally important to the above supplier considerations is an understanding of the key requirements associated with using peptones in a bioproduction process. First, a comprehensive peptone screen should be part of early medium development. Although peptones can be added at any point of that process — up to and through the bioreactor scale — generally the earlier that peptones are added so that the process can be optimized in their presence, the better the development outcome will be.

Simplifying the Screening Process

To identify the ideal peptone for an application, it is helpful to simplify the screening process. The following suggestions will enable rapid and efficient selection of peptones best suited to a process.

Begin with a solid, established basal medium designed for use with your cell line. Generally, a richer base medium will lead to faster, more successful project development results.

Screen and evaluate multiple products from the same peptone source substrate. Different peptones can have different compositions, so it is important to screen multiple products to identify which work best.

Develop a thorough experimental design to evaluate multiple concentrations. In addition, consider both individual and blended peptone conditions. This approach can help ensure that you find the optimal peptones and concentrations.

Characterize your process throughout using different analytical techniques, and establish baseline data through spent-media analysis. Examine different lots of peptones and base media to understand sources of variability to enable better consistency.

Evaluate proliferation, production, protein quality, and any other key attributes when conducting media and peptone screening. Monitoring

Figure 8: Optimization of peptone concentrations to achieve the desired charge variance profile

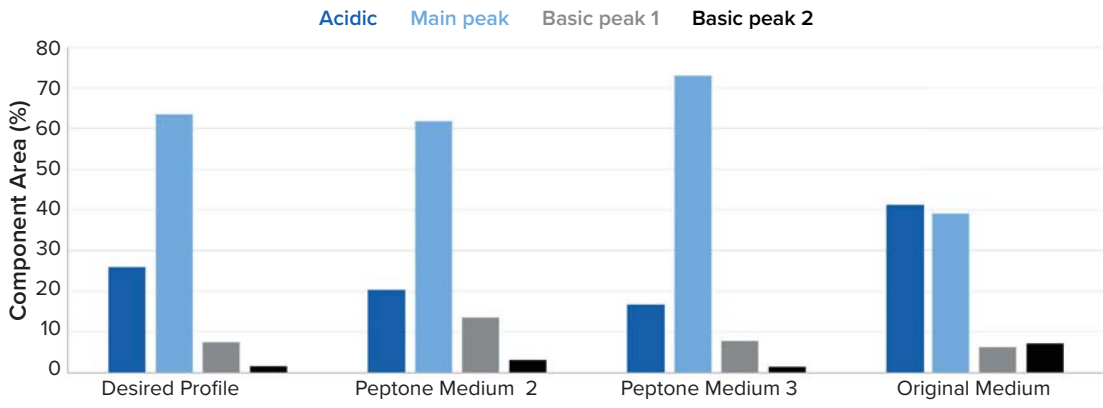
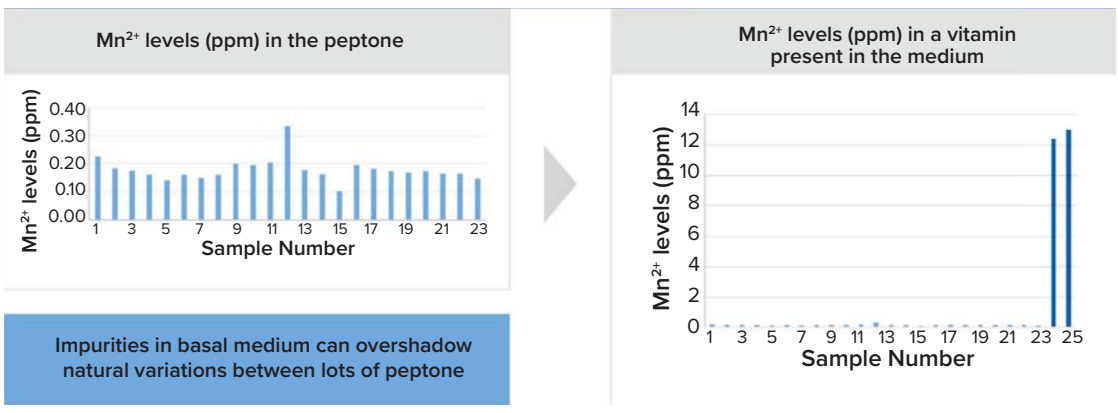


Figure 9: Manganese levels were assessed in 23 lots of peptone supplement and a vitamin present in the basal medium. The manganese component levels in the peptone were all <0.4 ppm, whereas the levels in the vitamin were >12 ppm in some samples. Comparison of levels in peptone lots (1–23) and vitamin lots (24–25) are shown in the right panel.



only one attribute will not predict overall performance accurately.

Identify key drivers of the specific bioproduction process and establish optimal ranges to achieve the desired target performance.

A Proven Bioprocess Strategy

Peptones are well-established supplements in the bioproduction industry with a proven track record in pharmaceuticals currently on the market, and they continue to be used in the development of next-generation biopharmaceutical innovations. The use of peptones in bioproduction processes provides a flexible, versatile, rapid, and efficient means of achieving desired targets in a wide range of cell types and processes.

Peptones are effective at improving cell growth, productivity, and product quality attributes including modulation of glycan profiles and charge variants. When considering and selecting peptones in a bioproduction project, it is essential 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 best peptones and a highly consistent bioproduction process.

Peptones have a proven history of success and continue to be incorporated in the development of future biotherapeutics.

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