

rProbumin™ AF (Recombinant Human Serum Albumin, Animal Free)

A Novel Cell Culture Supplement

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Abstract

Therapeutic protein production systems rely on the use of large quantities of cell culture media supplements such as serum albumin for promoting cell growth and increasing recombinant protein yield. The numerous functions of albumin include the maintenance of osmotic balance and an important role in the transport, distribution and metabolism of many endogenous and exogenous ligands (e.g. fatty acids, amino acids, steroids, metals and numerous pharmaceuticals). Advances in cell culture media development have allowed mammalian culture systems to gravitate towards an animal component-free growth environment. As the industry continues its move away from using animal-derived components, researchers look for alternative non-animal ingredients that can enhance cell culture performance. rProbumin AF (recombinant Human Serum Albumin, Animal Free), a structural equivalent to human serum albumin (HSA), was developed as an alternative to natural HSA and bovine-derived serum albumin (BSA) for cell culture applications. rProbumin AF is manufactured without animal- or human-derived raw materials ensuring no risk of contamination with viruses or other infectious agents known to be associated with these types of raw materials. Our research demonstrates that rProbumin AF supports growth and protein production in mammalian cell culture and is an effective replacement for traditional serum albumin.

Introduction

It is well established that the total elimination of animal-derived components from cell culture media is the most effective way of reducing the risk of contaminating biological processes and products with adventitious agents. rProbumin AF has been developed primarily for use in cell culture media formulations where there is a need to use animal-free ingredients without having to compromise cell culture performance. This includes the industrial cell-based production of biopharmaceuticals for human therapeutic use.

Celliance (now part of Millipore) and Novozymes A/S have entered into an exclusive agreement to develop and manufacture recombinant human serum albumin for use in cell culture applications. rProbumin AF is produced by recombinant DNA technology using a patented *Aspergillus* expression platform which Novozymes has used for more than 20 years to produce numerous food-grade enzymes. *Aspergillus* has a long history of safe industrial use and *Aspergillus*-derived recombinant proteins are GRAS (Generally Recognized as Safe). No human or animal-origin ingredients are used at any stage of rProbumin AF production, including the development and maintenance of the cell banks.

Our research demonstrates that rProbumin AF in cell culture supports growth and protein production in CHO cultures and is an effective replacement for traditional serum albumin from both human and bovine sources. The following data was generated with 3 pilot-scale development lots of rProbumin AF supplied as a liquid formulation.

Materials and Methods

Cell line and cell maintenance

CHO DXB11, a dihydrofolate reductase (dhfr-) Chinese Hamster Ovary fibroblast cell line, is commonly used in both industry and research platforms for selection of protein-expressing transfectants. For the studies presented, CHO DXB11 was cultured in a commercially available chemically defined, protein-free media (CDM) and Millipore's proprietary serum-free media (SFM) specifically designed for optimal suspension growth and protein production.

Growth Promotion and Protein Analysis

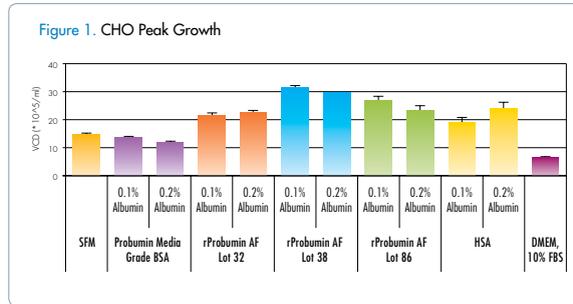
Exponentially growing cells were harvested, washed and seeded into duplicate shake flasks at 3×10^5 cells per flask in Millipore's proprietary SFM or commercial CDM with either 0.5g/L, 1g/L or 2g/L rProbumin AF as indicated. DMEM containing 10% FBS was used as the positive control. Growth and viability were measured by propidium-iodide uptake using a FACS growth promotion assay established at Millipore. Total IgG production was measured using an ELISA assay (Bethyl Labs) specific for the IgG being expressed.

Results

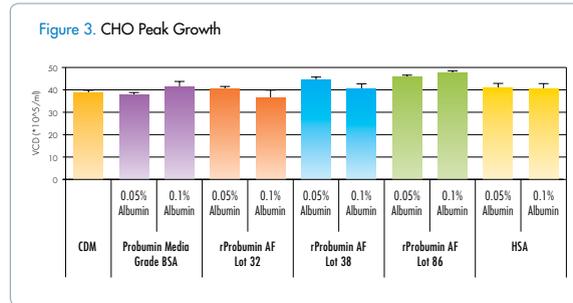
As shown in **Figure 1**, Millipore SFM supplemented with rProbumin AF at 1g/L or 2g/L (0.1% and 0.2% respectively) compared with the same concentrations of Probumin™ Media Grade BSA, (Millipore catalog #81-O68), and native human serum albumin (Millipore catalog #60001) exhibited an increase in growth promoting activity. As shown in **Figure 2**, the cumulative IgG level was much higher in the SFM supplemented with rProbumin AF compared with Probumin Media Grade BSA and native human serum albumin.

As observed in **Figure 3**, commercially available chemically-defined media (CDM) supplemented with rProbumin AF at 0.5g/L or 1g/L (0.05% and 0.1% respectively) compared with the same concentrations of Probumin Media Grade BSA and native human serum albumin exhibited a slight increase in growth promoting activity. As seen in **Figure 4**, an increase in the cumulative IgG level is observed in the commercial CDM supplemented with rProbumin AF compared with Probumin Media Grade BSA and native human serum albumin.

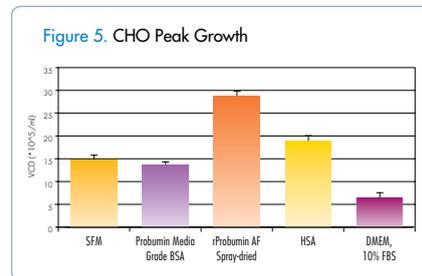
Presented in **Figures 5 and 6** are growth promotion and protein production data generated using a spray-dried powder version of rProbumin AF. Millipore SFM supplemented with rProbumin AF at 1g/L (0.1%) compared against the same concentration of Probumin Media Grade BSA and native human serum albumin resulted in an increase in growth promotion (**Figure 5**) and protein production (**Figure 6**) when cultures are grown in media containing the spray-dried form of rProbumin AF. Equivalent performance is observed in cultures grown in the Millipore SFM supplemented with either the solution or powder versions of rProbumin AF at 1g/L (data not shown).



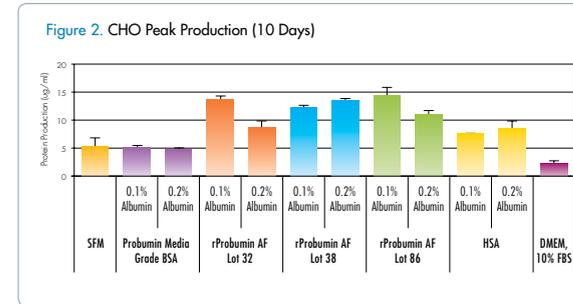
Cell density of CHO cultures grown in Millipore serum-free media (SFM) containing rProbumin AF compared to cultures grown in the same media containing Probumin Media Grade BSA and native human serum albumin. Increased cell density was observed in cultures grown with rProbumin AF.



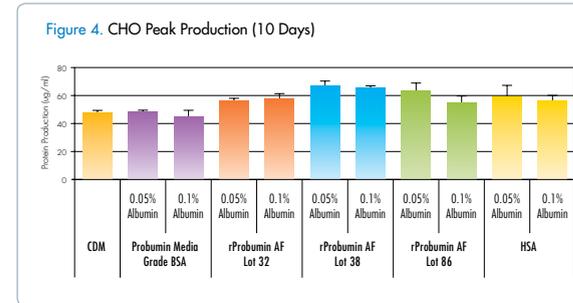
Cell density of CHO cultures grown in commercially available chemically-defined media (CDM) supplemented with rProbumin AF compared to cultures grown in the same media supplemented with Probumin Media Grade BSA and native human serum albumin. A slight increase in cell density was observed in cultures grown with rProbumin AF.



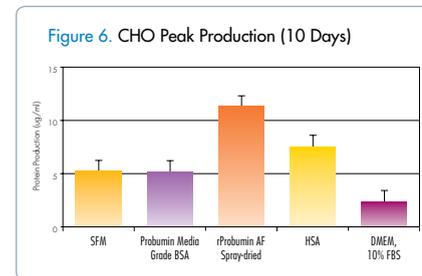
Cell density of CHO cultures grown in Millipore serum-free media containing 1g/L (0.1%) of a spray-dried form of rProbumin AF compared to cultures grown in the same media containing Probumin Media Grade BSA and native human serum albumin. Increased cell density was observed in cultures grown with rProbumin AF.



Cumulative IgG production in CHO cultures grown in Millipore serum-free media (SFM) containing rProbumin AF compared to the same media containing Probumin Media Grade BSA and native human serum albumin. Over 30% higher IgG production was observed in cultures grown with rProbumin AF compared to media containing Probumin Media Grade BSA and native human serum albumin.



Cumulative IgG production in CHO cultures grown in commercially available chemically-defined (CDM) media supplemented with rProbumin AF compared to cultures grown in the same media supplemented with Probumin Media Grade BSA and native human serum albumin. Increased IgG production was observed in cultures grown with rProbumin AF compared to the same media supplemented with Probumin Media Grade BSA and native human serum albumin.



Cumulative IgG production in CHO cultures grown in Millipore serum-free media containing 1g/L (0.1%) of a spray-dried form of rProbumin AF compared to the same media containing Probumin Media Grade BSA and native human serum albumin. Increased IgG production was observed in cultures grown with rProbumin AF compared to media containing Probumin Media Grade BSA and native human serum albumin.

rProbumin AF Fits Your Bioprocessing Needs

As shown in our experiments, media development and optimization or supplementation using rProbumin AF provides a way to increase cell proliferation and production by creating an optimal cell line specific culture environment.

- rProbumin AF is ideally suited for serum-free and/or chemically defined protein-free media development, optimization or supplementation.
- rProbumin AF exhibits equivalent or greater performance in growth promotion and protein production compared to traditional animal-derived albumin sources such as BSA and HSA. Supplementation at concentrations as low as 0.05% has been demonstrated to improve productivity.
- All cell lines tested could be transferred directly from medium containing BSA or HSA into media containing rProbumin AF. Consistent growth rate and protein production was maintained in subsequent subculture.
- rProbumin AF is produced without the use of any animal- or human-derived ingredients thereby eliminating concerns about the possible presence of adventitious contaminating agents or infectious prions.
- rProbumin AF is manufactured using a patented *Aspergillus* expression platform which has a long history of safe industrial use, particularly for the production of food grade enzymes.
- For customer convenience, a powder form of rProbumin AF is also under development.

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