A More Robust Bioprocessing Solution

rHSA

Recombinant Human Serum Albumin

Xeno-Free Albumin Specifically Developed for Enhancing Cell Culture Performance

ASPIRA SCIENTIFIC

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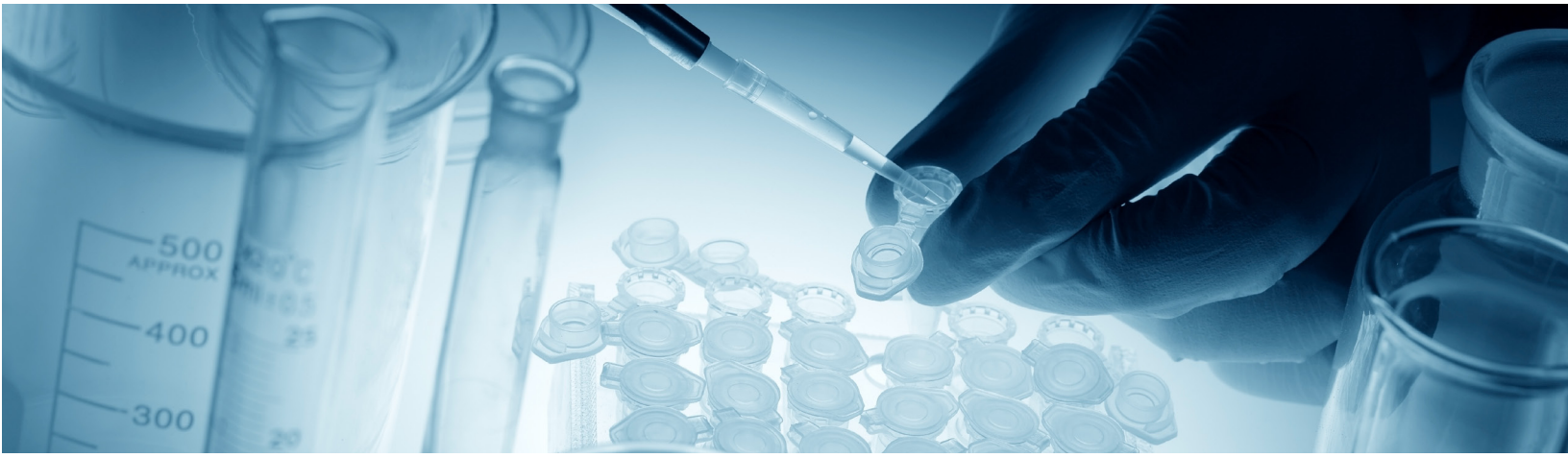

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Recombinant Human Serum Albumin (rHSA)

99%+ purity | Xeno-free | Expressed in rice

Albumin is a soluble, 66 kDa non-glycosylated monomeric protein. It is the most abundant protein in human blood and functions as an important carrier protein for fatty acids, steroids, hormones, growth factors, and trace minerals. It also functions to buffer pH, stabilize proteins and membranes, promote cell growth, protect from cellular damage, or serve as a hydrophobic moiety.

Human serum albumin (HSA) is a common ingredient in cell media formulations and has been successfully used for growing and storing cultured cells for stem cell, in vitro fertilization and other cell therapies. In cell culture, recombinant albumin is added as a supplement to media to increase the viability, productivity, and overall health of a wide range of cell types including embryonic stem cell, CHO, hybridoma, and fibroblast.

Albumin has traditionally been sourced from human or bovine serum. Due to increasing regulatory concerns over contaminants such as viruses, prions, and mycoplasma, batch-to-batch variability, and consistent supply, there is an increased demand for a more defined, animal-free replacement in the bioprocessing industry.

Aspira Scientific's recombinant HSA (rHSA) is a highly pure, non-glycosylated single polypeptide product that has an identical amino acid sequence and conforms to the biophysical characteristics of plasma-derived human serum albumin (pHSA). Furthermore, rHSA displays similar in vitro and in vivo immunogenicity as pHSA. Derived from rice grains, it does not utilize human, animal, or microorganism production systems, thus free of yeast, animal, and human byproducts and devoid of associated viral, prion, or other infectious contaminants.

Aspira Scientific's rHSA delivers all of the benefits of albumin without the regulatory obstacles related to FBS, BSA, and pHSA. Produced using a novel rice-based technology platform and manufactured in an ISO 9001 certified facility with rigorous quality control procedures and superior batch-to-batch consistency, Aspira Scientific's rHSA performs equally or better than pHSA or fetal bovine serum to support cell growth and conditions. It is an effective, risk-free, and more economical supplement for obtaining the best possible cell performance and improving viability in a chemically defined environment.

Having the same application fields as animal-derived serum, but the added advantages of high purity, xeno-free production, safety, low cost, and scale-up capabilities, Aspira Scientific's rHSA is the ideal choice for the most demanding research applications.

Optimize your cell culture strategy

Patented Rice-Expression System Allows for Efficient Scale-Up Production

Plasmids used for the expression of rHSA. The final transformed plasmid (Figure 1 bottom) originated from the expression plasmid (Figure 1 top).

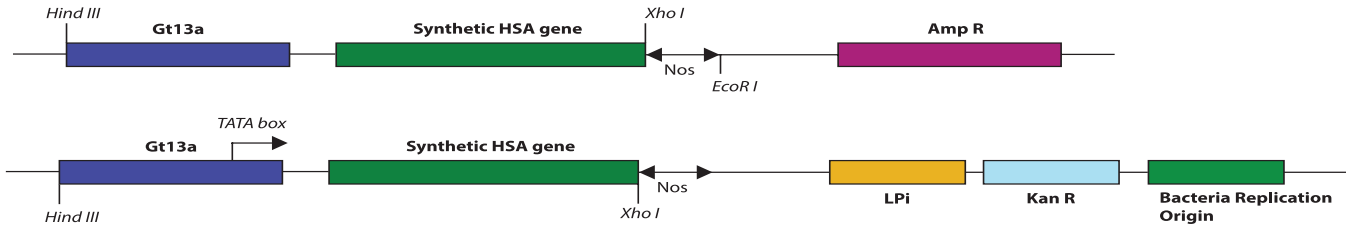
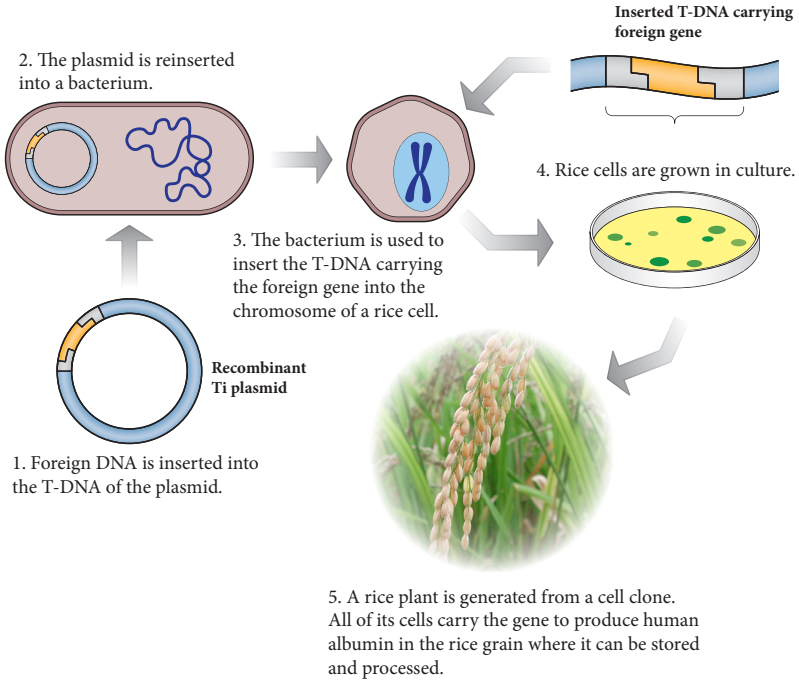


Figure 1

A 1,241-bp Gt13a promoter and signal peptide sequence (GenBank AP003256) were amplified from the rice genome of line TP 309 and fused into the mature HSA gene optimized with a rice codon bias. This gene was then synthesized with a SchI and an XhoI site at the 5′ and 3′ end, which was then cloned into a pOsPMP01 plasmid digested with NaeI/XhoI. The resulting construct, pOsPMP04, was digested with HindIII and EcoRI, and a 2,832-bp fragment was cloned into the binary vector pCambia1303 using the same enzyme sites. The resulting binary vector was designated pOsPMP114 and transformed into Agrobacterium strain EHA105.

Bulk production of highly purified rHSA is economically streamlined with a processing strategy that takes less than 3 days after harvesting the rice grains. After grinding the rice grains, three chromatography steps (Capto-MMC, Q-Sepharose and Phenyl-HP) are performed, followed by concentration/desalting, and lyophilization (Figure 2). Data from a scale-up purification showed that the recovery of rHSA was 55.75 ± 4%, equivalent to a yield of 2.75 g of rHSA per kilogram of rice, which is much higher than the cost-effective threshold of 0.1 g per kilogram typically observed in plants.



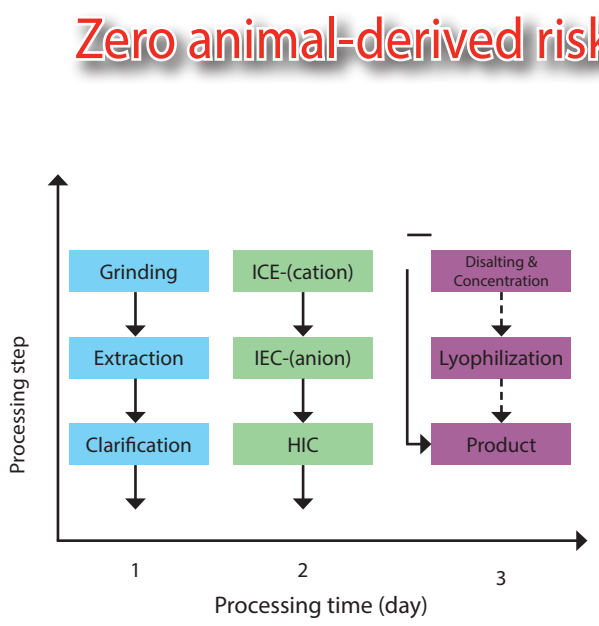
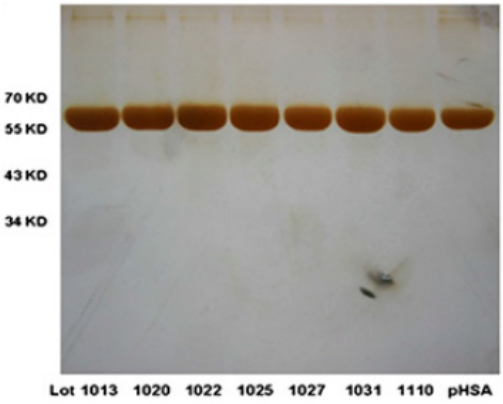


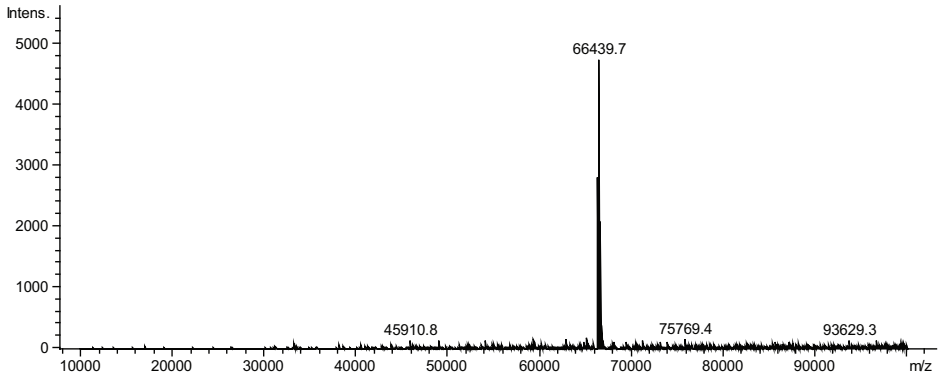
Figure 2

Biochemical & Biophysical Characterization

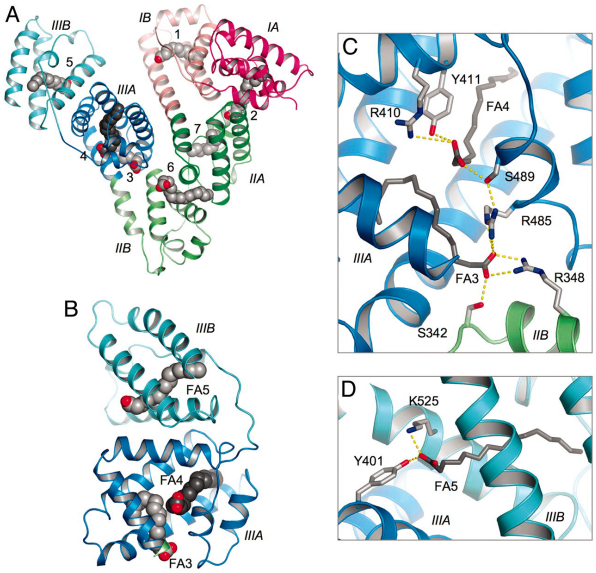
The molecular weight of rHSA and pHSA was determined by MALDI-TOF. rHSA was calculated to have a molecular weight of 69.367 kDa. pHSA was calculated to have a molecular weight of 66.439 kDa. The mass of pHSA and rHSA protein extract from rice seeds was assessed at dilution ratios of 1:500, 1:1,000, 1:2,000, and 1:4,000 by 12% SDS-PAGE analysis developed with 5-bromo-4-chloro-3-indolyl phosphate-nitrobluetetrazolium chloride. Molecular weights of pHSA was approximately 67 kDa and rHSA approximately 69 kDa.



A silver-stain SDS-PAGE gel showing Aspira Scientific's rHSA from large-scale production of different lot numbers, demonstrating the batch-to-batch consistency and comparability to pHSA.



The presence of modified forms of a protein, such as glycosylated or alkylated protein can cause serious effects on its functionality, as well as decreasing its usability in regulated studies. A detailed analysis from a triple quadrupole mass spectrometer confirms Aspira Scientific's rHSA as a single protein at 66.4 kDa that is representative of the native human serum albumin molecule. Peaks indicative of glycosylated or other posttranslational modified forms of the protein were not detected.

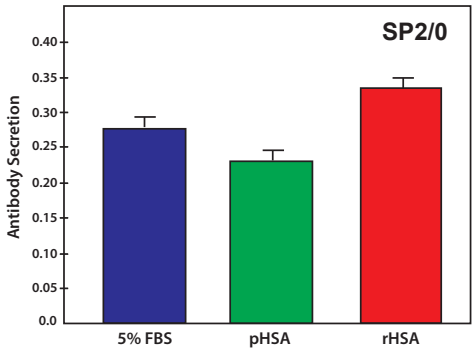


Structural details of full-length rHSA, domain III, and FA sites four and five as revealed by X-ray crystallography. Crystal structure of rHSA with palmitale bound (A) and domain III (B) from the same crystal structure, indicating the likely structural details of the recombinant fragment. rHSA contains three domains (I, red; II, green; III, blue), each containing A and B subdomains (dark- and light-colored shades, respectively). The crystal structure reveals seven LCFA-binding sites. The FA bound to site four has been darkened to distinguish it from the FA in site three. Shown also is the crystal structure of palmitate bound to FA sites four (C) and five (D). The electrostatic interactions formed between the FA carboxyl and basic residue(s) are indicated by the dashed yellow lines. FA bound to site four forms strong electrostatic interactions with R410 and Y411, whereas K525 forms stable interactions with FA in site five.

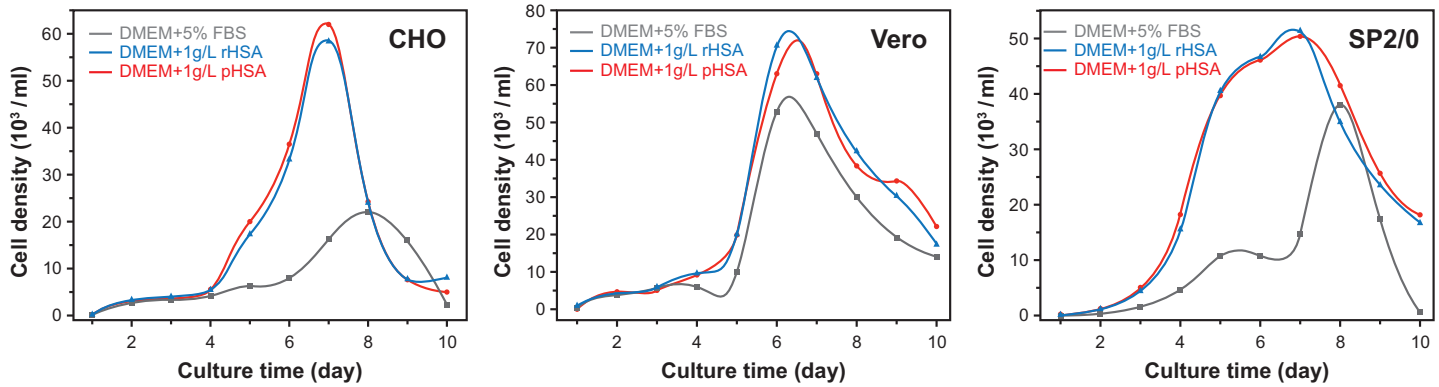
Unmatched purity and homogeneity

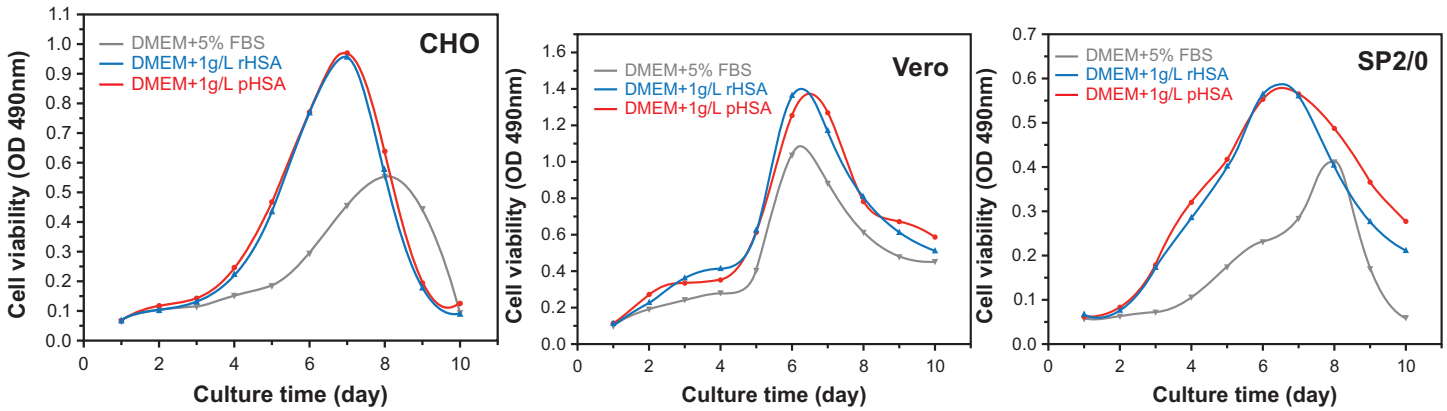
Performance of Recombinant Human Serum Albumin in Cell Culture

In cell culture, albumin is added to the media as a supplement to increase the growth and productivity of cells and improve overall cell health. Albumin transports important nutrients to cells, binds toxins to avoid negative growth conditions and damage to cells, and binds excessive proteins to act as a buffer and maintain stability. Albumin, as a cell culture supplement, has generally been sourced through bovine or human blood, but these sources have a number of associated problems such as reliability, variability, and biological contaminants. To evaluate the use of rHSA in media for cell growth promotion, CHO-K1, Vero and the hybridoma cell line SP2/0 were continuously cultured by media supplemented with FBS, pHSA, or Aspira Scientific's rHSA. All cell lines were seeded into a 96-well plate at a concentration of 5,000 cells/mL and cultured for 10 days under standard conditions (37°C, 5% CO_2). Cell lines were grown in standard DMEM supplemented with 5% FBS only (control), and with either 1 g/L rHSA or pHSA alone for all cell lines. Cell proliferation was determined by cell number count 24 hours after seeding and cell viability was assessed by a MTT assay.



Results:
This study highlights the benefits of using rHSA in a serum-free, chemically-defined media. Data indicates that media supplementation with rHSA improves CHO, Vero, and SP2/0 cell culture performance. Cell density and viability is significantly improved by the addition of rHSA to DMEM, and matches or even outperforms pHSA supplemented media. Antibody secretion of SP2/0 in batch suspension culture saw higher yields when grown in culture medium supplemented with rHSA rather than FBS or pHSA.





He, Y. et al. (2011). Proc Natl Acad Sci USA. 108(47), 19078-83

Ordering Information

Product	Size	SKU	Price USD
Recombinant Human Serum Albumin, rice-expressed	1 g	800001-1g	$120.00
Recombinant Human Serum Albumin, rice-expressed	5 g	800001-5g	$460.00
Recombinant Human Serum Albumin, rice-expressed	10 g	800001-10g	$785.00
Recombinant Human Serum Albumin, rice-expressed	100 g	800001-100g	$4,850.00
Recombinant Human Serum Albumin, rice-expressed	1 kg	800001-1kg	$35,000.00
Recombinant Human Serum Albumin, rice-expressed	1 mt	800001-bulk	Inquire

Growth Factors

Product	Size	SKU	Price USD
Recombinant Human Fibroblast GF, rice-expressed	10 µg	800002-10ug	$72.00
Recombinant Human Fibroblast GF, rice-expressed	50 µg	800002-50ug	$135.00
Recombinant Human Epidermal GF, yeast-expressed	100 µg	800004-100ug	$97.00

Specialty Proteins

Product	Size	SKU	Price USD
Recombinant Human Alpha-1 Antitrypsin, rice-expressed	1 mg	800003-1mg	$975.00
Recombinant Human Lactoferrin, rice-expressed	500 mg	800005-500mg	$195.00

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