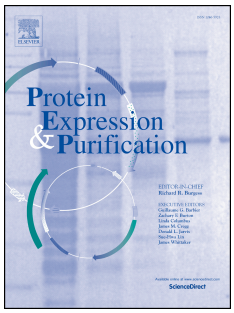
# Accepted Manuscript




High level expression and purification of recombinant human serum albumin in *Pichia pastoris*

Wen Zhu, Guihua Gong, Jie Pan, Shu Han, Wei Zhang, Youjia Hu, Liping Xie

PII: S1046-5928(17)30713-1

DOI: 10.1016/j.pep.2018.02.003

Reference: YPREP 5225

To appear in: *Protein Expression and Purification*

Received Date: 24 November 2017

Revised Date: 31 January 2018

Accepted Date: 8 February 2018

Please cite this article as: W. Zhu, G. Gong, J. Pan, S. Han, W. Zhang, Y. Hu, L. Xie, High level expression and purification of recombinant human serum albumin in *Pichia pastoris*, *Protein Expression and Purification* (2018), doi: 10.1016/j.pep.2018.02.003.

# High level expression and purification of recombinant human serum albumin in *Pichia pastoris*

Wen Zhu, Guihua Gong, Jie Pan, Shu Han, Wei Zhang, Youjia Hu, Liping Xie

China State Institute of Pharmaceutical Industry, Zhangjiang Institute, Shanghai 201203, PR China

## Abstract

Human serum albumin (HSA) has been extensively used in a series of clinical care settings for nearly seven decades. However, the broad application of this protein is seriously limited by its short supply. In this work, the codon sequence of HSA was cloned under the control of the alcohol oxidase 1 promoter (AOX1) and expressed as a secretory protein in *Pichia pastoris*. A recombinant strain displaying the highest HSA yield was selected by screening for resistance to the highest concentration of antibiotic G418. After optimizing the induction conditions and additional supplements, the highest yield of HSA reached 1.6 g/L in a shake flask. Performing high density fermentation further improved the highest yield to 8.86 g/L in a fermenter after 96 h of methanol induction. This result is more promising than the previous reports of industrial applications, which reported the highest yield as 92.29 mg/L/h, considering that the space-time yield of rHSA was doubled. In addition, the desired protein was purified by filtration and Cibacron Blue affinity chromatography, which yielded a 58% recovery of a product that had over a 96% purity. This study reveals that *Pichia pastoris* is an excellent system for recombinant human serum albumin expression due to its outstanding expression capacity. In addition, the high efficiency level of rHSA production lays a solid foundation for its use in industrial production.

Key word: Human serum albumin, *Pichia pastoris*, Secretory expression, Optimization, Space-time yield

## Introduction

As the major protein component of human plasma, human serum albumin (HSA) is utilized for innumerable *in vitro* studies and pharmaceutical applications [1]. The monomeric multi-domain macromolecule is a single nonglycosylated peptide chain made up of 585 amino acids (66.5 kDa) [2]. There are extensive applications of HSA, which including nanodelivery of drugs, vaccine formulation and manufacturing, cryopreservation, fusion of peptides, cell culture media and many other novel usages. The most significant application is as a blood volume expander [3]. The usual dosages of HSA are so high (over 10 g/dose) that the clinical demand for it are in excess of 500 metric tons worldwide in 2014 [4]. All of the medical grade HSA used is