ACM International Conference Proceeding Series
28 March 2019, Pages 289-293
9th International Conference on Biomedical Engineering and Technology, ICBET 2019; Meiji UniversityTokyo; Japan; 28 March 2019 through 30 March 2019; Code 149151

Expression and Purification of Soluble Bacterially-Expressed Human Hexokinase II in E.coli System (Conference Paper)

Tanbin, S., Fuad, F.A.A.

Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, PO Box 10, Kuala Lumpur, 50728, Malaysia

Abstract

View references (24)

Human hexokinase II (HKII) is one of the key enzymes in the glycolytic pathway. It has been postulated that HKII is a potential target for anti-dengue (DENV) drug development, as well as involved in cancer and tumor cell growth. In this work, the human hexokinase II (HKII) gene was cloned into pETite N-His SUMO vector and transformed into the E.coli strain HI-control 10G for the propagation of clones. Two different expression hosts, E.coli HI-control™ BL21 (DE3) and BL21 (DE3) pLysS were used to optimize HKII expression. In order to obtain the soluble recombinant HKII in a functional form, we optimized protein expression at three different temperatures; 17°C, 25°C and 37°C, at 24 hours incubation time. The soluble protein was expressed in the presence of 0.5 mM isopropyl-2-D-thiogalactopyranoside (IPTG) in TB media at 17°C for 24 hrs. The expressed protein was then purified to homogeneity by a combination of Immobilized Metal Ion Affinity Chromatography (IMAC), size exclusion chromatography (SEC) and ion-exchange chromatography (IEX), resulting in pure bacterially-expressed HK2. Taken together, this study has successfully produced soluble bacterially-expressed human HKII that can be utilized for further therapeutic studies. © 2019 Association for Computing Machinery.

Author keywords

Glycolysis | Human hexokinase II | Protein expression | Protein purification

Indexed keywords

Engineering controlled terms: Affinity chromatography | Biomedical engineering | Chromatographic analysis | Cloning | Controlled drug delivery | Ion chromatography | Ion exchange | Metal ions | Metals | Purification | Recombinant proteins

Engineering uncontrolled terms: Glycolysis | Glycolytic pathways | Hexokinase II | Immobilized metal ion affinity chromatographies | Ion exchange chromatography | Potential targets | Protein expressions | Protein purification

Engineering main heading: Size exclusion chromatography

ISBN: 978-145036130-9
Source Type: Conference Proceeding
Original language: English
DOI: 10.1145/3326172.3326219
Document Type: Conference Paper
Publisher: Association for Computing Machinery

1 Osawa, H., Printz, R.L., Whitesell, R.R., Granner, D.K.
Regulation of hexokinase II gene transcription and glucose phosphorylation by catecholamines, cyclic AMP, and insulin

(1995) *Diabetes*, 44 (12), pp. 1426-1432. Cited 44 times.
http://diabetes.diabetesjournals.org/
doi: 10.2337/diab.44.12.1426

View at Publisher

2 Jeong, E.-J., Park, K., Yi, S.Y., Kang, H.-J., Chung, S.J., Lee, C.-S., Chung, J.W., (...), Kim, M.
Stress-governed expression and purification of human type II Hexokinase in Escherichia coli

(2007) *Journal of Microbiology and Biotechnology*, 17 (4), pp. 638-643. Cited 5 times.

View at Publisher

3 Deeb, S.S., Malkki, M., Laakso, M.
Human hexokinase II: Sequence and homology to other hexokinases

(1993) *Biochemical and Biophysical Research Communications*, 197 (1), pp. 68-74. Cited 27 times.
doi: 10.1006/bbrc.1993.2442

View at Publisher

4 Printz, R.L., Ardehali, H., Koch, S., Granner, D.K.
Human hexokinase II mRNA and gene structure

(1995) *Diabetes*, 44 (3), pp. 290-294. Cited 43 times.
http://diabetes.diabetesjournals.org/
doi: 10.2337/diab.44.3.290

View at Publisher

5 Salans, L.B., Knittle, J.L., Hirsch, J.
The role of adipose cell size and adipose tissue insulin sensitivity in the carbohydrate intolerance of human obesity
(1968) *The Journal of Clinical Investigation*, 47 (1), pp. 153-165. Cited 336 times.

6 Liu, F., Dong, Q., Myers, A.M., Fromm, H.J.
Expression of human brain hexokinase in Escherichiacoli: Purification and characterization of the expressed enzyme

(1991) *Biochemical and Biophysical Research Communications*, 177 (1), pp. 305-311. Cited 31 times.
doi: 10.1016/0006-291X(91)91983-J

View at Publisher

7 Liu, H., Yang, H., Wang, X., Tu, Y.
The contribution of hexokinase 2 in glioma
(2018) *Cancer Translational Medicine*, 4 (2), pp. 54-58.

8 Kwee, S.A., Hernandez, B., Chan, O., Wong, L.
Choline Kinase Alpha and Hexokinase-2 Protein Expression in Hepatocellular Carcinoma: Association with Survival (Open Access)

(2012) *PLoS ONE*, 7 (10), art. no. e46591. Cited 57 times.
http://www.plosone.org/article/fetchObjectAttachment.action?uri=info%3Adoi%2F10.1371%2Fjournal.pone.0046591&representation=PDF
doi: 10.1371/journal.pone.0046591

View at Publisher

9 Machida, K., Ohta, Y., Osada, H.
Suppression of apoptosis by cyclophilin D via stabilization of hexokinase II mitochondrial binding in cancer cells (Open Access)

(2006) *Journal of Biological Chemistry*, 281 (20), pp. 14314-14320. Cited 100 times.
http://www.jbc.org/cgi/reprint/281/20/14314
doi: 10.1074/jbc.M513297200

View at Publisher

10 Pilkis, S.J., Weber, I.T., Harrison, R.W., Bell, G.I.
Glucokinase: Structural analysis of a protein involved in susceptibility to diabetes

(1994) *Journal of Biological Chemistry*, 269 (35), pp. 21925-21928. Cited 68 times.

View at Publisher

11 Aleshin, A.E., Fromm, H.J., Honzatko, R.B.
Multiple crystal forms of hexokinase I: New insights regarding conformational dynamics, subunit interactions, and membrane association

(1998) *FEBS Letters*, 434 (1-2), pp. 42-46. Cited 18 times.
doi: 10.1016/S0014-5793(98)00952-1

View at Publisher

12 Rosano, C., Sabini, E., Rizzi, M., Deriu, D., Murshudov, G., Bianchi, M., Serafini, G., (...), Bolognesi, M.
Binding of non-catalytic ATP to human hexokinase I highlights the structural components for enzyme-membrane association control (Open Access)

(1999) *Structure*, 7 (11), pp. 1427-1437. Cited 34 times.
http://www.journals.elsevier.com/structure/
doi: 10.1016/S0969-2126(00)80032-5

View at Publisher

13 Ruzzo, A., Andreoni, F., Magnani, M.
Structure of the human hexokinase type I gene and nucleotide sequence of the 5′ flanking region

(1998) *Biochemical Journal*, 331 (2), pp. 607-613. Cited 16 times.
www.biochemj.org
doi: 10.1042/bj3310607

View at Publisher

14 Bustamante, E., Pedersen, P.L.
High aerobic glycolysis of rat hepatoma cells in culture: Role of mitochondrial hexokinase (Open Access)

(1977) *Proceedings of the National Academy of Sciences of the United States of America*, 74 (9), pp. 3735-3739. Cited 306 times.
doi: 10.1073/pnas.74.9.3735

View at Publisher

15 Munger, J., Bajad, S.U., Coller, H.A., Shenk, T., Rabinowitz, J.D.
Dynamics of the cellular metabolome during human cytomegalovirus infection (Open Access)

(2006) *PLoS Pathogens*, 2 (12), pp. 1165-1175. Cited 183 times.
http://pathogens.plosjournals.org/archive/1553-7374/2/12/pdf/10.1371_journal.ppat.0020132-L.pdf
doi: 10.1371/journal.ppat.0020132

View at Publisher

16 Roe, B., Kensicki, E., Mohney, R., Hall, W.W.
Metabolomic profile of hepatitis C Virus-Infected hepatocytes (Open Access)

(2011) *PLoS ONE*, 6 (8), art. no. e23641. Cited 57 times.
doi: 10.1371/journal.pone.0023641

View at Publisher

17 Ritter, J.B., Wahl, A.S., Freund, S., Genzel, Y., Reichl, U.
Metabolic effects of influenza virus infection in cultured animal cells: Intra- and extracellular metabolite profiling (Open Access)

(2010) *BMC Systems Biology*, 4, art. no. 61. Cited 89 times.
http://www.biomedcentral.com/1752-0509/4/61
doi: 10.1186/1752-0509-4-61

View at Publisher

18 Vastag, L., Koyuncu, E., Grady, S.L., Shenk, T.E., Rabinowitz, J.D.
Divergent effects of human cytomegalovirus and herpes simplex virus-1 on cellular metabolism (Open Access)

(2011) *PLoS Pathogens*, 7 (7), art. no. e1002124. Cited 129 times.
http://www.plospathogens.org/article/fetchObjectAttachment.action?uri=info%3Adoi%2F10.1371%2Fjournal.ppat.1002124&representation=PDF
doi: 10.1371/journal.ppat.1002124

View at Publisher

19 Hollenbaugh, J.A., Munger, J., Kim, B.
Metabolite profiles of human immunodeficiency virus infected CD4+ T cells and macrophages using LC-MS/MS analysis (Open Access)

(2011) *Virology*, 415 (2), pp. 153-159. Cited 51 times.
doi: 10.1016/j.virol.2011.04.007

View at Publisher

20 Delgado, T., Sanchez, E.L., Camarda, R., Lagunoff, M.
Global Metabolic Profiling of Infection by an Oncogenic Virus: KSHV Induces and Requires Lipogenesis for Survival of Latent Infection (Open Access)

(2012) *PLoS Pathogens*, 8 (8), art. no. e1002866. Cited 69 times.
http://www.plospathogens.org/article/fetchObjectAttachment.action?uri=info%3Adoi%2F10.1371%2Fjournal.ppat.1002866&representation=PDF
doi: 10.1371/journal.ppat.1002866

View at Publisher

21 Fontaine, K.A., Camarda, R., Lagunoff, M.
Vaccinia virus requires glutamine but not glucose for efficient replication (Open Access)

(2014) *Journal of Virology*, 88 (8), pp. 4366-4374. Cited 45 times.
http://jvi.asm.org/content/88/8/4366.full.pdf
doi: 10.1128/JVI.03134-13

View at Publisher

22 Xiao, L., Hu, Z.-Y., Dong, X., Tan, Z., Li, W., Tang, M., Chen, L., (...), Cao, Y.
Targeting Epstein-Barr virus oncoprotein LMP1-mediated glycolysis sensitizes nasopharyngeal carcinoma to radiation therapy (Open Access)

(2014) *Oncogene*, 33 (37), pp. 4568-4578. Cited 76 times.
http://www.nature.com/onc/index.html
doi: 10.1038/onc.2014.32

View at Publisher

23 Fontaine, K.A., Sanchez, E.L., Camarda, R., Lagunoff, M.
Dengue virus induces and requires glycolysis for optimal replication
(2014) *Journal of Virology*, pp. JVI-02309. Cited 2 times.

24 Vasina, J.A., Baneyx, F.
Expression of aggregation-prone recombinant proteins at low temperatures: A comparative study of the Escherichia coli cspA and tac promoter systems

(1997) *Protein Expression and Purification*, 9 (2), pp. 211-218. Cited 111 times.
http://www.elsevier.com/inca/publications/store/6/2/2/9/3/5/index.htt
doi: 10.1006/prep.1996.0678

View at Publisher