J Appl Biotechnol Rep. 2025 December;12(4):1883-1886

Journal of
Applied Biotechnology Reports

doi 10.30491/jabr.2025.555733.1935

Short Communication

Applying the Alkaline Lysis Method for rProtein A Extraction from *E. coli* Bacteria: A Simple Approach for Large Protein Sample Extraction

Hamid Kooshki [1], Mozafar Mohammadi [2*], Mehdi Ebadi [2], Mohammad Sadegh Hashemzadeh [1]

[1] Nanobiotechnology Research Center, New Health Technologies Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
[2] Applied Biotechnology Research Center, New Health Technologies Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

Corresponding Author: Mozafar Mohammadi, PhD, Assistant Professor, Applied Biotechnology Research Center, New Health Technologies Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran. Tel: +989167379116, E-mail: mohammadi8390@gmail.com

Received October 27, 2025; **Accepted** November 10, 2025; **Online Published** December 30, 2025

Abstract

Introduction: The increasing demand for monoclonal antibodies in medicine requires scalable and efficient production methods. Because of its strong affinity for immunoglobulins, Staphylococcal protein A is a key tool for antibody purification. Conventional methods of extracting recombinant protein A (rProtein A), such as sonication, are costly, inefficient at high volumes, and difficult to scale up. Due to the inherent stability of rProtein A in alkaline pH, this study used NaOH to extract rProtein A from *E. coli*.

Materials and Methods: The protein was expressed in *E. coli BL21 (DE3)*, and then cell disruption was achieved through sonication and alkaline lysis. Ni-NTA affinity chromatography was employed to purify the isolated proteins. An ELISA-based assay evaluated the functionality and binding affinity of the purified rProtein A by determining the equilibrium dissociation constant (Kd).

Results: Alkaline lysis proved more effective, releasing nearly 98% of rProtein A from the cell pellet compared to 40% with sonication. Additionally, the functionality of rProtein A remained intact, exhibiting excellent antibody-binding affinity with a Kd of 6.95 nM, slightly better than the 7.04 nM Kd of the sonication-produced protein.

Conclusions: Overall, alkaline lysis is a reliable, cost-efficient, and scalable alternative to sonication for the primary recovery of rProtein A from bacterial cells.

Keywords: rProtein A, Monoclonal Antibody, Sonication, Alkaline Lysis, Protein Extraction

Citation: Kooshki H, Mohammadi M, Ebadi M, Hashemzadeh MS. Applying the Alkaline Lysis Method for rProtein A Extraction from *E. coli* Bacteria: A Simple Approach for Large Protein Sample Extraction. J Appl Biotechnol Rep. 2025;12(4):1883-1886. doi:10.30491/jabr.2025.555733.1935

Introduction

Due to the therapeutic effects of antibodies on viral diseases, cancer, rheumatic diseases, and other nervous system diseases, their production and purification have exponentially increased. For the purification of IgG, purified protein A is required to bind to the sorbent and capture IgG covalently.[1,2] Staphylococcal protein A is one of the first discovered immunoglobulin-binding proteins molecules and has been extensively studied during the past decades. Because of its affinity to immunoglobulins, protein A has been widely used as a tool in the detection and purification of antibodies and their role has been further developed in one of the most commonly employed affinity purification systems.[1-4]

Several methods are used to extract the rProtein A from bacterial cell pellets, such as sonication and high-pressure French press methods. However, these methods are expensive and only applicable to low volumes of samples.[5,6] Due to the acceptable resistance of Protein A toward harsh acidic and basic cleaning conditions,[2,7] it could be extracted using NaOH alkaline lysis. In this study, we used NaOH to extract the rProtein A from *E. coli* and compared it with the sonication method as a standard approach for bacterial cell disruption.

Materials and Methods

Construction and Cloning of the rProtein A Plasmid

The coding sequence for the Protein A construct was synthesized by Shine Gene Company (China) and cloned into the pET-28a(+) expression vector (Novagen, USA). The calcium chloride ($CaCl_2$) approach was used to create competent *E. coli BL21 (DE3)* cells. For transformation, 5 µl of the pET-28a(+) vector with the Protein A insert was carefully combined with 50 µl of competent cells. To stabilize the cells, the mixture was incubated on ice for 30 minutes, then subjected to a heat shock at 42 °C for 90 seconds. Finally, it was chilled on ice for 5 minutes, and then 800 µl of fresh Luria-Bertani (LB) broth (Quelab,

Canada) was added, and the culture was shaken at 180 rpm for 2 hours at 37 °C. After that, transformed cells were plated on LB agar (Quelab, Canada) supplemented with 50 µg/ml of kanamycin (Molekula, UK) and incubated at 37 °C for 20 hours.

Protein Expression

Single colonies, confirmed by PCR, were cultivated overnight in 10 ml of LB broth supplemented with kanamycin (50 µg/ml) at 37 °C with shaking at 180 rpm. The overnight culture was then diluted 1:100 into 1 L fresh LB medium and incubated until the optical density at 600 nm (OD_{600}) reached between 0.6 and 0.8. To induce protein expression, 0.1 mM IPTG (Sigma, USA) was added, and the culture was kept at 37 °C with shaking at 180 rpm for an additional 16 hours. Afterward, cells were collected by centrifugation at 8,000 rpm for 10 minutes. The cell pellets were preserved at −20 °C.

Protein Extraction Using NaOH

First, for every milligram of cell pellet, 5-10 ml of phosphate-buffered saline (PBS buffer) is added, followed by 0.1 M NaOH (Final concentration), and incubated for 5 minutes at room temperature. Then, the mixture is fully homogenized using a rotary homogenizer (Heidolph, Germany). The homogenized mixture is centrifuged for 10 minutes at 10,000 rpm at 4 °C. Subsequently, the pH of the supernatant is adjusted to 8 with HCl while the sample is being stirred, and then centrifuged for 30-60 minutes at 12,000 rpm at 4 °C to precipitate other host cell proteins and debris.

Using a Probe Sonicator

The sonicator (UP400S Hielscher, Germany), which operated at 90% amplitude with 10 cycles of 20-second ON and 30-second OFF pulses, was used to lyse cell suspensions. After that, the resultant lysate was centrifuged for 20 minutes at 14,000 rpm to separate the soluble proteins. After being cleared, the supernatant was collected and stored at 4 °C until purification.

Protein Purification

Ni-NTA affinity chromatography (Arg Biotech, Iran) was used to purify the His-tagged proteins. PBS supplemented with 10 mM imidazole (Merck, Germany) was used as the binding buffer to pre-equilibrate the column. To effectively eliminate nonspecific impurities, the resin was thoroughly washed with 30 mM imidazole in PBS after loading the samples. The target proteins were eluted using PBS containing 300 mM imidazole. Following purification, the proteins were dialyzed into PBS buffer for use in the subsequent process.

Protein Function Assessment Using ELISA

100 µl of human antibody at a concentration of 10 µg/ml was added to the wells of a 96-well ELISA plate in triplicate (except for the negative control) and incubated overnight in a refrigerator at 4 °C. After washing the wells with PBST (PBS + 0.1% Tween 20), 300 µl of blocking solution (5% skimmed milk in PBST) was added, and the wells were kept at room temperature for one hour. Then, they were washed five times, and concentrations of 0.1, 1, 10, and 100 nanomolar of purified protein A were added to the wells. The ELISA plate (Maxwell, China) was incubated for one hour at room temperature. After washing the plate, 100 µl of anti-His-tag HRP-conjugated antibody (Cyto Matin Gene, Iran) (diluted 1:1000 in blocking solution) was added and incubated for one hour at room temperature. Finally, after washing the plate, 100 µl of TMB buffer was added to the wells, and after 15 minutes of incubation at room temperature in a dark environment, the dye reaction was stopped by 50 µl of 0.2 M sulfuric acid, and the optical absorption was read at a wavelength of 450 nm. GraphPad Prism 10.5.0[8] was used to calculate the dissociation constants (Kd).

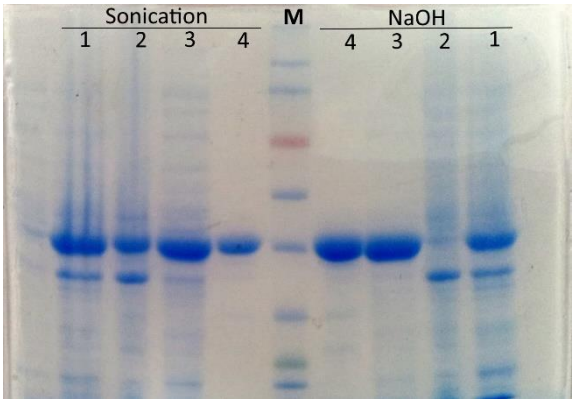

Figure 1. SDS PAGE Analysis of Protein Extraction Efficiency Using Sonication and NaOH Methods. Lane 1: Crude lysate, Lane 2: Bacterial cell pellet, Lane 3: Supernatant, Lane 4: Purified protein A, and Lane M: Protein ladder.

Results

The pET-28a(+) expression vector containing the recombinant *protein A* gene was successfully transformed into *E. coli* bacteria, and expression was optimized. Next, both sonication and alkaline lysis methods were used to extract protein from the bacteria. ImageJ software was used to measure the density of the protein bands in the SDS PAGE test. As shown in Figure 1, the efficiency of the alkaline lysis method in destroying the bacterial cell wall and extracting protein was better than sonication. In the alkaline lysis method, about 98 percent of protein A is released from the bacteria, and almost no protein A remains in the bacterial pellet, whereas approximately 60 percent of protein A remains in the pellet and is not released when using sonication. Additionally, because NaOH destroys most bacterial proteins, they do not appear in the supernatant as seen in lane 3 (Figure 1).

When examining protein–ligand interactions, being able to measure binding affinities via equilibrium dissociation constants (Kd) is essential for understanding the nature of the complexes formed.[9] To evaluate and compare the function and antibody binding efficiency of extracted protein A by sonication and alkaline lysis methods, the ELISA technique was used to determine the dissociation constants (Kd). As shown in Figure 2, Kd values of 7.04 and 6.95 nM were obtained for the sonication and alkaline lysis methods, respectively. As a result, the affinity levels obtained from both methods did not differ significantly.

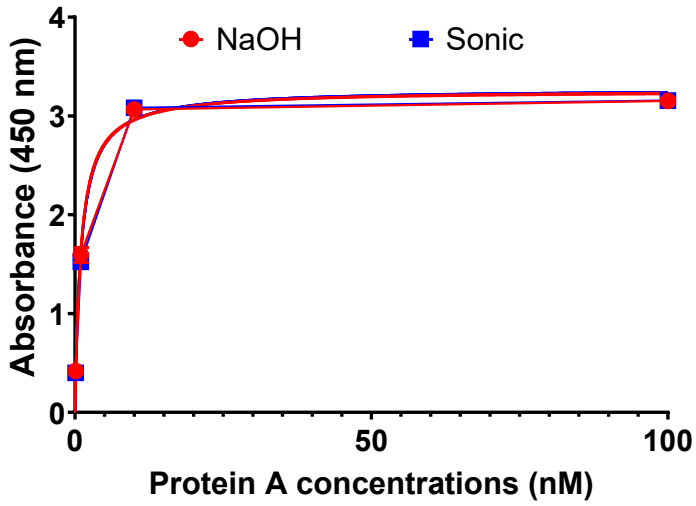

Figure 2. Determination of Binding Affinity (Kd) by ELISA. A saturation binding curve measuring the interaction between Protein A and antibody.

Discussion

Because of its affinity to immunoglobulins, protein A has been widely used as a tool in the detection and Purification of antibodies.[1,10,11] Several methods are used to extract the rProtein A from bacterial cell pellets, such as sonication and high-pressure French press methods. However, these methods are expensive and only applicable to low volumes of samples.[5,6]

Protein A can tolerate a pH range of 2 to 13 and maintain its function.[2,7] For this reason, using alkaline conditions with NAOH, the bacteria expressing protein A can be lysed well and extracted with high efficiency. As demonstrated in this work, the alkaline lysis method surpasses the sonication method in several aspects. First, a larger amount of bacterial pellet can be lysed in a shorter time. Second, the alkaline lysis method can be performed at a lower cost.

As the results of this study showed, many bacterial proteins were degraded due to their instability in response to pH changes and were not present in the final supernatant. This makes the purification results obtained by both chromatographic and non-chromatographic methods excellent. Furthermore, the use of the alkaline lysis method did not have any negative impact on the function of rProtein A or its antibody binding ability. As shown in the ELISA test, the affinity of rProtein A obtained by alkaline lysis was slightly better. Some of the rProtein A may have been damaged during sonication.

The manufacture of recombinant therapeutic proteins, including monoclonal antibodies, requires the removal of endotoxins (Lipopolysaccharides, LPS) due to their strong immunogenicity and the strict regulatory constraints placed on their use in clinical settings.[12,13] To guarantee product safety and avoid patients experiencing negative inflammatory reactions, effective clearance is crucial.[14,15] Another advantage of using NaOH could be degradation and removal of bacterial endotoxin. Using the alkaline method to extract protein A may help to remove endotoxin more effectively during the process of producing recombinant drugs. Because of some limitations we couldn't validate this hypothesis however it should be evaluated in the next works.

Conclusion

Overall, alkaline lysis is a reliable, cost-efficient, and scalable alternative to sonication for the primary recovery of rProtein A from bacterial cells. Finally, it is recommended that this method be used to extract proteins that are resistant to alkaline conditions. Some proteins can even be genetically engineered to be resistant to alkali while maintaining their efficiency and function, so that alkaline lysis can be used to extract them from the bacterial host.

Authors' Contributions

All authors contributed equally to this study.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

References

1. Zarrineh M, Mashhadi IS, Farhadpour M, Ghassempour A. Mechanism of antibodies purification by protein A. Anal Biochem. 2020;609:113909. doi:10.1016/j.ab.2020.113909
2. Ramos-de-la-Peña AM, González-Valdez J, Aguilar O. Protein A chromatography: Challenges and progress in the purification of monoclonal antibodies. J Sep Sci. 2019;42(9):1816-27. doi:10.1002/jssc.201800963
3. Hober S, Nord K, Linhult M. Protein A chromatography for antibody purification. J Chromatogr B. 2007;848(1):40-7. doi:10.1016/j.jchromb.2006.09.030
4. Kangwa M, Yelemane V, Ponnurangam A, Fernández-Lahore M. An engineered *Staphylococcal* Protein A based ligand: Production, characterization and potential application for the capture of Immunoglobulin and Fc-fusion proteins. Protein Expr Purif. 2019;155:27-34. doi:10.1016/j.pep.2018.11.003
5. Tripathi NK, Shrivastava A. Recent developments in bioprocessing of recombinant proteins: expression hosts and process development. Front Bioeng Biotechnol. 2019;7:420. doi:10.3389/fbioe.2019.00420
6. Singh RS. A comparative study on cell disruption methods for release of aspartase from *E. coli* K-12. Indian J Exp Biol. 2013;51(11):997-1003.
7. Wetterhall M, Ander M, Björkman T, Musunuri S, Palmgren R, Rodrigo G. Investigation of alkaline effects on Protein A affinity ligands and resins using high resolution mass spectrometry. J Chromatogr B. 2021;1162:122473. doi:10.1016/j.jchromb.2020.122473
8. GraphPad Prism version 10.5.0 for Windows, GraphPad Software, La Jolla California USA. Available from: www.graphpad.com
9. Housden NG, Kleanthous C. Thermodynamic dissection of colicin interactions. Methods Enzymol. 2011;488:123-45. doi:10.1016/B978-0-12-381268-1.00006-9
10. Shukla AA, Thömmes J. Recent advances in large-scale production of monoclonal antibodies and related proteins. Trends Biotechnol. 2010;28(5):253-61. doi:10.1016/j.tibtech.2010.02.001
11. Amritkar V, Adat S, Tejwani V, Rathore A, Bhambure R. Engineering *Staphylococcal* Protein A for high-throughput affinity purification of monoclonal antibodies. Biotechnol Adv. 2020;44:107632. doi:10.1016/j.biotechadv.2020.107632
12. Schneier M, Razdan S, Miller AM, Briceno ME, Barua S. Current technologies to endotoxin detection and removal for biopharmaceutical purification. Biotechnol Bioeng. 2020;117(8):2588-609. doi:10.1002/bit.27362
13. Ritzén U, Rotticci-Mulder J, Strömberg P, Schmidt SR. Endotoxin reduction in monoclonal antibody preparations using arginine. J Chromatogr B. 2007;856(1-2):343-7. doi:10.1016/j.jchromb.2007.06.020
14. Magalhães PO, Pessoa Jr. A. Lipopolysaccharide, LPS removal, Depyrogenation. Encyclopedia of Industrial Biotechnology. 2025. pp. 1-6.
15. Hatlem D, Christensen M, Broeker NK, Kristiansen PE, Lund R, Barbirz S, et al. A trimeric coiled-coil motif binds bacterial lipopolysaccharides with picomolar affinity. Front Cell Infect Microbiol. 2023;13:1125482. doi:10.3389/fcimb.2023.1125482