PEMURNIAN PROTEIN NANOBODI KORTISOL DARI *Escherichia coli* BL21 (DE3) DENGAN METODE *IMMOBILIZED METAL AFFINITY CHROMATOGRAPHY* DALAM KONDISI NATIF DAN DENATURASI

SKRIPSI

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Pembimbing	Pembimbing
	
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ABSTRAK

Nanobodi (Nb) merupakan suatu antibodi yang dapat mendeteksi kortisol, proses penyiapan Nb sebagai agen pendeteksi memerlukan berbagai tahapan salah satunya pemurnian. *Immobilized metal affinity chromatography* (*IMAC*) menjadi metode yang memanfaatkan *histag* pada Nb untuk memperoleh Nb dengan kemurnian tinggi. Pemurnian dapat dilakukan pada kondisi natif maupun denaturasi karena *histag* tidak memerlukan konformasi yang spesifik untuk berikatan dengan Ni-NTA (*Nikel-Nitrilotriacetic acid*). Penelitian ini bertujuan untuk memperoleh Nb murni dengan kadar optimum berdasarkan SDS-PAGE dan uji *Bradford* serta mengetahui pengaruh perbedaan kondisi pemurnian pada kondisi natif dan denaturasi. Penelitian ini diawali dengan transformasi pET28a-Nb mutan pada inang *E. coli* BL21 (DE3), ekspresi protein Nb dengan induksi IPTG, pemurnian menggunakan metode *IMAC*, konfirmasi hasil dengan SDS-PAGE dan uji *Bradford*. Hasil penelitian menunjukan bahwa keberadaan Nb murni ditunjukan dengan pita tunggal berukuran 11-17 kDa berdasarkan SDS-PAGE, kadar optimum diperoleh pada kondisi denaturasi sebanyak 211,286 dan 224,143 ppm sedangkan kondisi natif sebesar 152,714 ppm. Kondisi pemurnian yang berbeda memberikan pengaruh terhadap kemudahan *histag* untuk berikatan dengan Ni-NTA, protein dalam bentuk kumparan rantai polipeptida (kondisi denaturasi) dapat meningkatkan kemampuan pengikatan *histag* dengan Ni-NTA dibandingkan dalam bentuk terlipat (kondisi natif). Kondisi denaturasi memberikan perolehan protein Nb murni dengan jumlah yang lebih banyak dibandingkan pemurnian pada kondisi natif.

Kata kunci: nanobodi, pemurnian, IMAC, kondisi natif, kondisi denaturasi.

ABSTRACT

Nanobody (Nb) is an antibody that can detect cortisol, the process of preparing Nb as a detection agent requires various stages including purification. Immobilized metal affinity chromatography (IMAC) is a method that utilizes the histag on Nb to obtain Nb with high purity. Purification can be done in native or denaturation conditions as the histag does not require a specific conformation to bind to the Ni-NTA (Nikel-Nitrilotriacetic acid). The research is aimed at obtaining pure Nb at optimal rates based on SDS-PAGE and Bradford tests as well as finding out the impact of differences in purification conditions on native and denaturation conditions. The research began with transformation of pET28a-Nb mutant in E. coli BL21 (DE3), Nb protein expression with IPTG induction, purification using IMAC method, confirmation of results with SDS-PAGE and Bradford test. The results showed that the existence of pure Nb is demonstrated with a single band 11-17 kDa based on SDS-PAGE, optimum rates obtained in denaturation conditions of 211,286 and 224,143 ppm while native conditions of 152,714 ppm. Different purification conditions influence the histage's ease of binding to Ni-NTA, protein in the form of polypeptide chain (denaturation condition) can enhance the binding capacity of histages to Ni-NTA compared to in folding form (kondisi natif). The denaturation condition provides the acquisition of pure Nb protein in larger quantities than purification in native conditions.

Keywords: *nanobody, purification, IMAC, native condition, denaturing condition.*

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DAFTAR ISI

DAFTAR TABEL

DAFTAR GAMBAR

DAFTAR LAMPIRAN

DAFTAR PUSTAKA

Abidin, A., Rahim, R.A., Arshad, M., Nabilah, M., Voon, C., Tang, T. dan Citartan, M. (2017) 'Current and Potential Developments of Cortisol Aptasensing towards Point-of-Care Diagnostics (POTC)', *Sensors 2017, Vol. 17, Page 1180*, 17(5), p. 1180. https://doi.org/10.3390/S17051180.

Angelousi, A., Margioris, A.N. dan Tsatsanis, C. (2020) 'ACTH Action on the Adrenals', *Endotext.* https://www.ncbi.nlm.nih.gov/books/NBK279118/ (Diakses pada: 11 Januari 2024).

Asyhari, M. H., Palupi, N. S., & Faridah, D. N. (2018). Karakteristik kimia konjugat isolat protein kedelai-laktosa yang berpotensi dalam penurunan alergenisitas. *Jurnal Teknologi & Industri Pangan*, 29(1).

Baghban, R., Gargari, S.L.M., Rajabibazl, M., Nazarian, S. dan Bakherad, H. (2016) 'Camelid-derived heavy-chain nanobody against *Clostridium botulinum* neurotoxin E in *Pichia pastoris'*, *Biotechnology and applied biochemistry*, 63(2), pp. 200–205. https://doi.org/10.1002/BAB.1226.

Barakat, S., Berksöz, M., Zahedimaram, P., Piepoli, S. dan Erman, B. (2022) 'Nanobodies as molecular imaging probes', *Free Radical Biology and Medicine*, 182, pp. 260–275. https://doi.org/10.1016/J.FREERADBIOMED.2022.02.031.

Baroroh, U., Setiani, N.A., Mardiah, I., Astriany, D. dan Yusuf, M. (2022) 'Computational Design of Nanobody Binding to Cortisol to Improve Their Binding Affinity Using Molecular Docking and Molecular Dynamics Simulations', *Indonesian Journal of Chemistry*, 22(2), pp. 515–525. https://doi.org/10.22146/IJC.71480.

Bartnicki, F., Kowalska, E., Pels, K., & Strzalka, W. (2015). Imidazole-free purification of His3-tagged recombinant proteins using ssDNA aptamer-based affinity chromatography. *Journal of chromatography* A, 1418, 130.

Bhatwa, A., Wang, W., Hassan, Y. I., Abraham, N., Li, X. Z., & Zhou, T. (2021). Challenges associated with the formation of recombinant protein inclusion bodies in Escherichia coli and strategies to address them for industrial applications. *Frontiers in Bioengineering and Biotechnology*, 9, 630551.

Botía, M., Escribano, D., Martínez-Subiela, S., Tvarijonaviciute, A., Tecles, F., López-Arjona, M. and Cerón, J.J. (2023) 'Different Types of Glucocorticoids to Evaluate Stress and Welfare in Animals and Humans: General Concepts and Examples of Combined Use', *Metabolites*, 13(1). https://doi.org/10.3390/METABO13010106.

Campuzano, S., Salema, V., Moreno-Guzmán, M., Gamella, M., Yáñez-Sedeño, P., Fernández, L.A. dan Pingarrón, J.M. (2014) 'Disposable amperometric magnetoimmunosensors using nanobodies as biorecognition element.

Determination of fibrinogen in plasma', *Biosensors and Bioelectronics*, 52, pp. 255–260. https://doi.org/10.1016/J.BIOS.2013.08.055.

Chang, Y., Zhang, X., Murchie, A. I., & Chen, D. (2022). Transcriptome profiling in response to Kanamycin B reveals its wider non-antibiotic cellular function in Escherichia coli. *Frontiers in Microbiology*, 13, 937827.

Chen, J., Luo, H., Tao, M., Liu, Z., & Wang, G. (2021). Quantitation of nucleoprotein complexes by uv absorbance and bradford assay. *Biophysics Reports*, 7(6), 429.

Cheung, R.C.F., Wong, J.H. dan Ng, T.B. (2012) 'Immobilized metal ion affinity chromatography: a review on its applications', *Applied microbiology and biotechnology*, 96(6), pp. 1411–1420. https://doi.org/10.1007/S00253-012-4507-0.

Crauwels, M., Van Vaerenbergh, N., Kulaya, N.B., Vincke, C., D'Huyvetter, M., Devoogdt, N., Muyldermans, S. dan Xavier, C. (2020) 'Reshaping nanobodies for affinity purification on protein a', *New biotechnology*, 57, pp. 20–28. https://doi.org/10.1016/J.NBT.2020.01.005.

De Meyer, T., Muyldermans, S. dan Depicker, A. (2014) 'Nanobody-based products as research and diagnostic tools', *Trends in Biotechnology*, 32(5), pp. 263–270.: https://doi.org/10.1016/j.tibtech.2014.03.001.

Ding, L., Wang, Z., Zhong, P., Jiang, H., Zhao, Z., Zhang, Y., Ren, Z. dan Ding, Y. (2019) 'Structural insights into the mechanism of single domain VHH antibody binding to cortisol', *FEBS Letters*, 593(11), pp. 1248–1256. https://doi.org/10.1002/1873-3468.13398.

Dmitriev, O.Y., Lutsenko, S. dan Muyldermans, S. (2016) 'Nanobodies as probes for protein dynamics in vitro and in cells', *Journal of Biological Chemistry*, 291(8), pp. 3767–3775. https://doi.org/10.1074/jbc.R115.679811.

Dziurkowska, E. dan Wesolowski, M. (2021) 'Cortisol as a Biomarker of Mental Disorder Severity', *Journal of Clinical Medicine*, 10(21), p. 5204. https://doi.org/10.3390/JCM10215204.

Fan, R., Du, J., Park, K.W., Chang, L.H., Strieter, E.R. dan Andrew, T.L. (2021) 'Immobilization of Nanobodies with Vapor-Deposited Polymer Encapsulation for Robust Biosensors', *ACS applied polymer materials*, 3(5), pp. 2561–2567. https://doi.org/10.1021/ACSAPM.1C00140.

Ferrer-Miralles, N., Domingo-Espín, J., Corchero, J., Vázquez, E. dan Villaverde, A. (2009) 'Microbial factories for recombinant pharmaceuticals', *Microbial Cell Factories*, 8(1), pp. 1–8. https://doi.org/10.1186/1475-2859-8-17/FIGURES/3.

Fong, B.A., Wu, W.Y. dan Wood, D.W. (2010) 'The potential role of self-cleaving purification tags in commercial-scale processes', *Trends in biotechnology*, 28(5), pp. 272–279. https://doi.org/10.1016/J.TIBTECH.2010.02.003.

Francis DM, Page R (2010) Strategies to optimize protein expression in E. coli. *Curr Protoc Protein Sci Chapter 5:Unit* 5 24 1–29. doi: https://doi.org/10.1002/0471140864.ps0524s61

Goldring, J. D. (2015). Spectrophotometric methods to determine protein concentration. *Western Blotting: Methods and Protocols*, 41-47.

Gonzalez-Sapienza, G., Rossotti, M.A. dan Tabares-da Rosa, S. (2017) 'Single-domain antibodies as versatile affinity reagents for analytical and diagnostic applications', *Frontiers in Immunology*, 8(AUG), p. 288027. https://doi.org/10.3389/FIMMU.2017.00977/BIBTEX.

Green, M. R., & Sambrook, J. (2019). Analysis of DNA by agarose gel electrophoresis. *Cold Spring Harbor Protocols,* 2019(1), pdb-top100388.

Guckeisen, T., Hosseinpour, S., & Peukert, W. (2021). Effect of pH and urea on the proteins secondary structure at the water/air interface and in solution. *Journal of Colloid and Interface Science*, 590, 38-49.

Guo, K., Wustoni, S., Koklu, A., Díaz-Galicia, E., Moser, M., Hama, A., Alqahtani, A.A., Ahmad, A.N., Alhamlan, F.S., Shuaib, M., Pain, A., McCulloch, I., Arold, S.T., Grünberg, R. dan Inal, S. (2021) 'Rapid single-molecule detection of COVID-19 and MERS antigens via nanobody-functionalized organic electrochemical transistors', *Nature biomedical engineering*, 5(7), pp. 666–677. https://doi.org/10.1038/S41551-021-00734-9.

He, Z., Tan, J. S., Lai, O. M., & Ariff, A. B. (2015). Optimization of conditions for the single step IMAC purification of miraculin from Synsepalum dulcificum. *Food chemistry*, 181, 19-24.

Hussack, G., Hirama, T., Ding, W., MacKenzie, R. dan Tanha, J. (2011) 'Engineered single-domain antibodies with high protease resistance and thermal stability', *PloS one*, 6(11). https://doi.org/10.1371/JOURNAL.PONE.0028218.

Ischak, Netty Ino. (2014). Isolasi DNA dan Protein dengan Teknik PCR, Elektroforesis Agarose dan SDS-PAGE. *Jurnal Entropi* Vol 9. PP. 751-757.

Jeong, H., Kim, H.J. dan Lee, S.J. (2015) 'Complete Genome Sequence of *Escherichia coli* Strain BL21', *Genome Announcements*, 3(2), pp. 134–149. https://doi.org/10.1128/GENOMEA.00134-15.

Jin, B.K., Odongo, S., Radwanska, M. dan Magez, S. (2023) 'Nanobodies: A Review of Generation, Diagnostics and Therapeutics', *International journal of molecular sciences*, 24(6). https://doi.org/10.3390/IJMS24065994.

Kadmiel, M. dan Cidlowski, J.A. (2013) 'Glucocorticoid receptor signaling in health and disease', *Trends in pharmacological sciences*, 34(9), pp. 518–530. https://doi.org/10.1016/J.TIPS.2013.07.003.

Karakus, C., Uslu, M., Yazici, D. dan Salih, B.A. (2016) 'Evaluation of immobilized metal affinity chromatography kits for the purification of histidine-tagged recombinant CagA protein', *Journal of Chromatography B*, 1021, pp. 182–187. https://doi.org/10.1016/J.JCHROMB.2015.11.045.

Kariuki, C.K. dan Magez, S. (2021) 'Improving the yield of recalcitrant Nanobodies® by simple modifications to the standard protocol', *Protein Expression and Purification*, 185, p. 105906. https://doi.org/10.1016/J.PEP.2021.105906.

Katrahalli, U., Kalanur, S.S. dan Seetharamappa, J. (2010) 'Interaction of bioactive coomassie brilliant blue g with protein: insights from spectroscopic methods', *Scientia pharmaceutica*, 78(4), pp. 869–880. https://doi.org/10.3797/SCIPHARM.1008-15.

Kaushik, A., Yndart, A., Jayant, R.D., Sagar, V., Atluri, V., Bhansali, S. dan Nair, M. (2015) 'Electrochemical sensing method for point-of-care cortisol detection in *human immunodeficiency virus*-infected patients', *International Journal of Nanomedicine*, 10, pp. 677–685. https://doi.org/10.2147/IJN.S75514.

Kemenkes RI. 2020. Farmakope Indonesia Edisi VII. Kementerian Kesehatan Republik Indonesia.

Khirehgesh, M. R., Sharifi, J., Safari, F., & Akbari, B. (2021). Immunotoxins and nanobody-based immunotoxins: Review and update. *Journal of drug targeting,* 29(8), 848-862

Kielkopf, C.L., Bauer, W. dan Urbatsch, I.L. (2020) 'Purification of Polyhistidine-Tagged Proteins by Immobilized Metal Affinity Chromatography', *Cold Spring Harbor protocols*, 2020(6), pp. 205–216. https://doi.org/10.1101/PDB.PROT102194.

Kim, S., Jeong, H., Kim, E.Y., Kim, J.F., Lee, S.Y. dan Yoon, S.H. (2017) 'Genomic and transcriptomic landscape of *Escherichia coli* BL21(DE3)', *Nucleic acids research*, 45(9), pp. 5285–5293. https://doi.org/10.1093/NAR/GKX228.

Kinna, A., Tolner, B., Rota, E.M., Titchener-Hooker, N., Nesbeth, D. dan Chester, K. (2016) 'IMAC capture of recombinant protein from unclarified mammalian cell feed streams', *Biotechnology and bioengineering*, 113(1), pp. 130–140. https://doi.org/10.1002/BIT.25705.

Koklu, A., Wustoni, S., Guo, K., Silva, R., Salvigni, L., Hama, A., Diaz-Galicia, E., Moser, M., Marks, A., McCulloch, I., Grünberg, R., Arold, S.T. dan Inal, S. (2022) 'Convection Driven Ultrarapid Protein Detection via Nanobody-Functionalized Organic Electrochemical Transistors', *Advanced materials (Deerfield Beach, Fla.)*, 34(35). https://doi.org/10.1002/ADMA.202202972.

Kong, Q., Yao, Y., Chen, R. dan Lu, S. (2014) 'Progress in nanobody and its application in diagnosis.', *Sheng wu gong cheng xue bao = Chinese journal of biotechnology*.

Krishnarjuna, B., & Ramamoorthy, A. (2022). Detergent-free isolation of membrane proteins and strategies to study them in a near-native membrane environment. *Biomolecules*, 12(8), 1076.

L, T., J, G. dan S, S. (2019) 'Physiology, Cortisol', *StatPearls*. http://europepmc.org/books/NBK538239 (Diakses pada: 10 Januari 2024).

Lebendiker M, Danieli T, de Marco A (2014) The Trip Adviser guide to the protein science world: a proposal to improve the awareness concerning the quality of recombinant proteins. *BMC Res Notes* 7:585. https://doi.org/10.1186/1756-0500-7-585

Lee, D.Y., Kim, E. dan Choi, M.H. (2015) 'Technical and clinical aspects of cortisol as a biochemical marker of chronic stress', *BMB reports*, 48(4), pp. 209–216. https://doi.org/10.5483/BMBREP.2015.48.4.275.

Lee S.C., Knowles T.J., Postis V.L., Jamshad M., Parslow R.A., Lin Y.P., Goldman A., Sridhar P., Overduin M., Muench S.P., et al. (2016). A method for detergent-free isolation of membrane proteins in their local lipid environment. Nat. *Protoc*. 2016;11:1149–1162. doi: 10.1038/nprot.2016.070

Liang, L., Hu, Z., Huang, Yingying, Duan, S., He, J., Huang, Yong, Zhao, Y. dan Lu, X. (2016) 'Advances in Nanobodies', *Journal of Nanoscience and Nanotechnology*, 16(12), pp. 12099–12111. https://doi.org/10.1166/JNN.2016.13767.

Liu, X., Liu, L., Wang, Y., Wang, X., Ma, Y., & Li, Y. (2014). The Study on the factors affecting transformation efficiency of E. coli competent cells. *Cell*, 5.

López-Laguna, H., Voltà-Durán, E., Parladé, E., Villaverde, A., Vázquez, E. dan Unzueta, U. (2022) 'Insights on the emerging biotechnology of histidine-rich peptides', *Biotechnology Advances*, 54, p. 107817. https://doi.org/10.1016/J.BIOTECHADV.2021.107817.

Malhotra, A. (2009) 'Tagging for protein expression', *Methods in enzymology*, 463(C), pp. 239–258. https://doi.org/10.1016/S0076-6879(09)63016-0.

Maria Bintang, (2018) 'Biokimia teknik penelitian'. https://lib.ui.ac.id (Diakses pada: 11 Januari 2024).

Marisch, K., Bayer, K., Cserjan-Puschmann, M., Luchner, M. dan Striedner, G. (2013) 'Evaluation of three industrial *Escherichia coli* strains in fed-batch cultivations during high-level SOD protein production', *Microbial Cell Factories*, 12(1), pp. 1–11. https://doi.org/10.1186/1475-2859-12-58/FIGURES/5.

McEwen, B.S. (2019) 'What Is the Confusion with Cortisol?', *Chronic Stress*, 3. https://doi.org/10.1177/2470547019833647.

Michels, A. dan Michels, N. (2014) 'Addison Disease: Early Detection and Treatment Principles', *American Family Physician*, 89(7), pp. 563–568. https://www.aafp.org/pubs/afp/issues/2014/0401/p563.html (Diakses pada: 11 Januari 2024).

Mir, M.A., Mehraj, U., Sheikh, B.A. dan Hamdani, S.S. (2020) 'Nanobodies: The "Magic Bullets" in therapeutics, drug delivery and diagnostics.', *Human Antibodies*, 28(1), pp. 29–51. https://doi.org/10.3233/HAB-190390.

Mitchell, L.S. dan Colwell, L.J. (2018) 'Analysis of nanobody paratopes reveals greater diversity than classical antibodies', *Protein Engineering, Design and*

Selection, 31(7–8), pp. 267–275. https://doi.org/10.1093/PROTEIN/GZY017.

Mohseni, A., Molakarimi, M., Taghdir, M., Sajedi, R.H. dan Hasannia, S. (2019) 'Exploring single-domain antibody thermostability by molecular dynamics simulation', *Journal of biomolecular structure & dynamics*, 37(14), pp. 3686–3696. https://doi.org/10.1080/07391102.2018.1526116.

Nakamura, T., Koma, D., Oshima, M., Hoshino, H., Ohmoto, T., & Uegaki, K. (2018). Application of chromosomal gene insertion into Escherichia coli for expression of recombinant proteins. *Journal of bioscience and bioengineering*, 126(2), 266-272.

Noble, J. E. (2014). Quantification of protein concentration using UV absorbance and Coomassie dyes. *In Methods in enzymology* (Vol. 536, pp. 17-26). *Academic Press*.

Oakley, R.H. dan Cidlowski, J.A. (2013) 'The biology of the glucocorticoid receptor: new signaling mechanisms in health and disease', *The Journal of allergy and clinical immunology*, 132(5), pp. 1033–1044. https://doi.org/10.1016/J.JACI.2013.09.007.

Oliveira, C., & Domingues, L. (2018). Guidelines to reach high-quality purified recombinant proteins. *Applied microbiology and biotechnology*, 102, 81-92

Pavani, P., Kumar, K., Rani, A., Venkatesu, P., & Lee, M. J. (2021). The influence of sodium phosphate buffer on the stability of various proteins: Insights into protein-buffer interactions. *Journal of Molecular Liquids*, 331, 115753.

Pavlova, A. S., Dyudeeva, E. S., Kupryushkin, M. S., Amirkhanov, N. V., Pyshnyi, D. V., & Pyshnaya, I. A. (2018). SDS-PAGE procedure: Application for characterization of new entirely uncharged nucleic acids analogs. *Electrophoresis*, 39(4), 670-674.

Pardon, E., Laeremans, T., Triest, S., Rasmussen, S.G.F., Wohlkönig, A., Ruf, A., Muyldermans, S., Hol, W.G.J., Kobilka, B.K. dan Steyaert, J. (2014) 'A general protocol for the generation of Nanobodies for structural biology', *Nature protocols*, 9(3), p. 674. https://doi.org/10.1038/NPROT.2014.039.

Purwanto, M.G.M. (2014) 'Perbandingan Analisa Kadar Protein Terlarut dengan berbagai Metode Spektroskopi UV-Visible'.

Qiagen (2011) *Sample & Assay Technologies QIAexpress ® Ni-NTA Fast Start Handbook*. www.qiagen.com.

Rahimzadeh, M., Sadeghizadeh, M., Najafi, F., Arab, S., & Mobasheri, H. (2016). Impact of heat shock step on bacterial transformation efficiency. *Molecular biology research communications*, 5(4), 257.

Ramamoorthy, S. dan Cidlowski, J.A. (2016) 'Corticosteroids: Mechanisms of Action in Health and Disease', *Rheumatic diseases clinics of North America*, 42(1), pp. 15–31. https://doi.org/10.1016/J.RDC.2015.08.002.

Rekowski, A., Langenkämper, G., Dier, M., Wimmer, M. A., Scherf, K. A., & Zörb, C. (2021). Determination of soluble wheat protein fractions using the Bradford assay. *Cereal Chemistry*, 98(5), 1059-1065.

Rodrigues, R.C., Ortiz, C., Berenguer-Murcia, Á., Torres, R. dan Fernández-Lafuente, R. (2013) 'Modifying enzyme activity and selectivity by immobilization', *Chemical Society Reviews*, 42(15), pp. 6290–6307. https://doi.org/10.1039/C2CS35231A.

Rosano, G.L. dan Ceccarelli, E.A. (2014) 'Recombinant protein expression in *Escherichia coli*: advances and challenges', *Frontiers in microbiology*, 5(APR). https://doi.org/10.3389/FMICB.2014.00172.

Rötzschke, O., Lau, J. M., Hofstätter, M., Falk, K., & Strominger, J. L. (2002). A pH-sensitive histidine residue as control element for ligand release from HLA-DR molecules. *Proceedings of the National Academy of Sciences*, 99(26), 16946-16950.

Saffarian, P., Peerayeh, S. N., Amani, J., Ebrahimi, F., Sedighianrad, H., Halabian, R., & Imani Fooladi, A. A. (2016). Expression and purification of recombinant TAT-BoNT/A (1–448) under denaturing and native conditions. *Bioengineered*, 7(6), 478-483.

Salvador, J.P., Vilaplana, L. dan Marco, M.P. (2019) 'Nanobody: outstanding features for diagnostic and therapeutic applications', *Analytical and bioanalytical chemistry*, 411(9), pp. 1703–1713. https://doi.org/10.1007/S00216-019-01633-4.

Schlager, B., Straessle, A., & Hafen, E. (2012). Use of anionic denaturing detergents to purify insoluble proteins after overexpression. *BMC biotechnology*, 12.

Schlegel S., Hjelm A., Baumgarten T., Vikström D., de Gier J.W. Bacterial-based membrane protein production. *BBA-Mol. Cell Res*. 2014;1843:1739–1749. doi: 10.1016/j.bbamcr.2013.10.023.

Schoch, C.L., Ciufo, S., Domrachev, M., Hotton, C.L., Kannan, S., Khovanskaya, R., Leipe, D., McVeigh, R., O'Neill, K., Robbertse, B., Sharma, S., Soussov, V., Sullivan, J.P., Sun, L., Turner, S. dan Karsch-Mizrachi, I. (2020) 'NCBI Taxonomy: a comprehensive update on curation, resources and tools', *Database: The Journal of Biological Databases and Curation*, 2020. https://doi.org/10.1093/DATABASE/BAAA062.

Setiani, N.A., Baroroh, U., Pia Handayani, A., Chairunnisa, N. dan Mardiah, I. (2022) *Prosiding Seminar Nasional Diseminasi Hasil Penelitian Program Studi S1 Farmasi Konstruksi Plasmid Rekombinan pET-28a-Nanobodi Secara in Silico*. https://bioinformatics.org/.

Sha, J., Wang, Y., Wang, J., Ren, L., Tu, Q., Liu, W., ... & Wang, J. (2011). Capillary-composited microfluidic device for heat shock transformation of Escherichia coli. *Journal of bioscience and bioengineering*, 112(4), 373-378.

Shanehbandi, D., Saei, A. A., Zarredar, H., & Barzegari, A. (2013). Vibration and glycerol-mediated plasmid DNA transformation for Escherichia coli. *FEMS microbiology letters*, 348(1), 74-78

Sheng, Y., Wang, K., Lu, Q., Ji, P., Liu, B., Zhu, J., Liu, Q., Sun, Y., Zhang, J., Zhou, E.M. dan Zhao, Q. (2019) 'Nanobody-horseradish peroxidase fusion protein as an ultrasensitive probe to detect antibodies against *Newcastle disease virus* in the immunoassay', *Journal of Nanobiotechnology*, 17(1), pp. 1–15. https://doi.org/10.1186/S12951-019-0468-0/FIGURES/7.

Shilling, P. J., Mirzadeh, K., Cumming, A. J., Widesheim, M., Köck, Z., & Daley, D. O. (2020). Improved designs for pET expression plasmids increase protein production yield in Escherichia coli. *Communications biology*, 3(1), 214.

Simas, R. G., Pessoa Junior, A., & Long, P. F. (2023). Mechanistic aspects of IPTG (isopropylthio-β-galactoside) transport across the cytoplasmic membrane of Escherichia coli—a rate limiting step in the induction of recombinant protein expression. *Journal of Industrial Microbiology and Biotechnology*, 50(1).

Soltaninasab, S., Ahmadzadeh, M., Shahhosseini, S., & Mohit, E. (2022). Evaluating the efficacy of immobilized metal affinity chromatography (IMAC) for host cell protein (HCP) removal from anti-HER2 scFv expressed in Escherichia coli. *Protein Expression and Purification*, 190, 106004

Spriestersbach, A., Kubicek, J., Schäfer, F., Block, H. dan Maertens, B. (2015) 'Purification of His-Tagged Proteins', *Methods in Enzymology*, 559, pp. 1–15. https://doi.org/10.1016/BS.MIE.2014.11.003.

Su, Q., Shi, W., Huang, X., Yin, S., Yang, X. dan Lu, X. (2023) 'Recent advances of nanobody applications in diagnosis and detection', *MedComm – Biomaterials and Applications*, 2(3). https://doi.org/10.1002/MBA2.54.

Sugiyono. (2016). Metode Penelitian Kuantitatif, Kualitatif, dan Kombinasi (Mixed Methods). Bandung: Alfabeta.

Sun, S., Ding, Z., Yang, X., Zhao, X., Zhao, M., Gao, L., Chen, Q., Xie, S., Liu, A., Yin, S., Xu, Z. dan Lu, X. (2021) 'Nanobody: A Small Antibody with Big Implications for Tumor Therapeutic Strategy', *International Journal of Nanomedicine*, 16, p. 2337. https://doi.org/10.2147/IJN.S297631.

Ta, D.T., Steen Redeker, E., Billen, B., Reekmans, G., Sikulu, J., Noben, J.P., Guedens, W. dan Adriaensens, P. (2015) 'An efficient protocol towards site-specifically clickable nanobodies in high yield: cytoplasmic expression in *Escherichia coli* combined with intein-mediated protein ligation', *Protein engineering, design & selection: PEDS*, 28(10), pp. 351–363. https://doi.org/10.1093/PROTEIN/GZV032.

Thau, L., Gandhi, J. dan Sharma, S. (2023) 'Physiology, Cortisol', *StatPearls* https://www.ncbi.nlm.nih.gov/books/NBK538239/ (Diakses pada: 11 Januari 2024).

Tripathi, N.K. dan Shrivastava, A. (2019) 'Recent Developments in Bioprocessing of Recombinant Proteins: Expression Hosts and Process Development',

Frontiers in Bioengineering and Biotechnology, 7. https://doi.org/10.3389/FBIOE.2019.00420.

Turner, R., Joseph, A., Titchener-Hooker, N., & Bender, J. (2018). Manufacturing of proteins and antibodies: chapter downstream processing technologies. *New Bioprocessing Strategies: Development and Manufacturing of Recombinant Antibodies and Proteins*, 95-114

Vercoe, P., & Well, A. (2020). Gel Electrophoresis. *Definitions*. https://doi.org/10.1007/978-3-642-11274-4_2477.

Wagner, H.J., Wehrle, S., Weiss, E., Cavallari, M. dan Weber, W. (2018) 'A Two-Step Approach for the Design and Generation of Nanobodies', *International Journal of Molecular Sciences 2018, Vol. 19, Page 3444*, 19(11), p. 3444. https://doi.org/10.3390/IJMS19113444.

Walsh, G. (2018) 'Biopharmaceutical benchmarks 2018', *Nature Biotechnology 2018 36:12*, 36(12), pp. 1136–1145. https://doi.org/10.1038/nbt.4305.

Walsh, G. dan Walsh, E. (2022) 'Biopharmaceutical benchmarks 2022', *Nature Biotechnology 2022 40:12*, 40(12), pp. 1722–1760. https://doi.org/10.1038/s41587-022-01582-x.

Wang, Y., Li, X., Chen, X., Nielsen, J., Petranovic, D. dan Siewers, V. (2021) 'Expression of antibody fragments in *Saccharomyces cerevisiae* strains evolved for enhanced protein secretion', *Microbial cell factories*, 20(1). https://doi.org/10.1186/S12934-021-01624-0.

Xu, H., Bao, X., Hong, W., Wang, A., Wang, K., Dong, H., Hou, J., Govinden, R., Deng, B. dan Chenia, H.Y. (2021) 'Biological Characterization and Evolution of Bacteriophage T7-△holin During the Serial Passage Process', *Frontiers in Microbiology*, 12, p. 705310. https://doi.org/10.3389/FMICB.2021.705310/BIBTEX.

Xu, L., Song, X. dan Jia, L. (2017) 'A camelid nanobody against EGFR was easily obtained through refolding of inclusion body expressed in *Escherichia coli'*, *Biotechnology and applied biochemistry*, 64(6), pp. 895–901. https://doi.org/10.1002/BAB.1544.

Yong Joon Kim, J., Sang, Z., Xiang, Y., Shen, Z. dan Shi, Y. (2023) 'Nanobodies: Robust miniprotein binders in biomedicine', *Advanced Drug Delivery Reviews*, 195, p. 114726. https://doi.org/10.1016/J.ADDR.2023.114726.

Zare, H., Aghamollaei, H., Hosseindokht, M., Heiat, M., Razei, A. dan Bakherad, H. (2021) 'Nanobodies, the potent agents to detect and treat the Coronavirus infections: A systematic review', *Molecular and Cellular Probes*, 55, p. 101692. https://doi.org/10.1016/J.MCP.2020.101692.

Zhang, Y., Wang, Y., & Li, Y. (2019). Major cause of antibody artifact bands on non-reducing SDS-PAGE and methods for minimizing artifacts. *Protein expression and purification*, 164, 105459.