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# In Silico Development of a Multi-Epitope Vaccine Using Advanced Bioinformatics Tools and Techniques

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## Abstract

The rapid global spread and impact of SARS-CoV-2 and other emerging infectious diseases highlight the urgent need for the swift development of effective vaccines. Traditional vaccine production processes are time-consuming and resource-intensive. The present study focused on an in-silico strategy combined with sophisticated bioinformatics tools to develop a multi-epitope vaccine candidate against the SARS-CoV-2 membrane glycoprotein. The full SARS-CoV-2 genome was downloaded from NCBI to determine the sequence of the membrane glycoprotein for further research. The selected protein was subjected to antigenicity and non-allergenicity testing with the assistance of computational tools. Immuno-informatics software was used to predict B-cell and T-cell epitopes, like MHC-I and MHC-II sequences. The predicted epitopes' binding affinity to the respective MHC alleles and their ability to cover different populations were evaluated. Through a systematic in silico approach, we identified potential immunogenic epitopes that resulted in constructing a multi-epitope vaccine construct to generate strong immune responses. The present research demonstrates how bioinformatics accelerates vaccine development while minimizing the need for extensive initial experimental validation.

**Keywords:** SARS-CoV-2, Multi-epitope vaccine, In silico design, Bioinformatics, B-cell epitopes, T-cell epitopes, Membrane glycoprotein, Immuno-informatics

## Introduction

The SARS-CoV-2 pandemic is a century-level global health emergency highlighting the importance of effective preventive measures such as vaccines (Krammer *et al.*, 2020). The manufacture of traditional vaccines entails time-consuming steps of antigen discovery and purification, subsequent weakening or inactivation, and rigorous laboratory and animal safety testing (Plotkin *et al.,* 2011). The appearance of novel virus variations highlights the requirement for quick and adaptable vaccine production methods (Harvey *et al*., 2021). In silico vaccinology offers a productive substitute for conventional vaccine development methods through the use of bioinformatics databases and resources (Rappuoli *et al.,* 2016). It facilitates the identification of vaccine candidates and immunogenic epitopes that streamline development by minimizing the need for early-stage laboratory and animal tests, cutting costs (De Groot *et al.,* 2004). Advantages of in silico vaccine design include rapid identification and synthesis of vaccine candidates, a necessity during pandemics, and its cost-effectiveness, safety, and allows individualized vaccine design based on population genetics or emerging viral strains (Flower *et al.,* 2010). By utilizing the potential of bioinformatics tools and databases, in silico vaccinology provides a new and complementary platform to traditional approaches (Ong *et al.,* 2020). The approach enables the discovery of candidate vaccines and immunogenic epitopes in silico, hence streamlining the development process, lowering the costs, and eradicating large-scale laboratory and animal-based testing in the initial phase. Multi-epitope vaccines with the rationally engineered immunogenic epitopes of the antigenic target hold the enormous potential to generate broad and robust immunity (Chauhan *et al.,* 2021). SARS-CoV-2 membrane (M) glycoprotein is a viral assembly, budding, and envelope formation-associated structural protein and therefore an excellent antigen to be engineered as a vaccine (Thomas, 2020). The current study employed an integrated in silico strategy to design a multi-epitope vaccine construct targeting the SARS-CoV-2 membrane glycoprotein.

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## Materials and Methods

*Integrated Framework for In Silico Vaccine Design*

The Insilco vaccine design undergoes several steps that include sequence retrieval of the desired protein, B cells & T cells epitope prediction from the sequence retrieved, population coverage analysis, vaccine construction, structural modeling, and validation, including secondary structure analysis, tertiary structure prediction, refinement and validation, molecular docking and simulation, codon optimization and in silico cloning, and immune simulation.

*Retrieval of Sequence and Evaluation of Its Antigenicity & Allergenicity*

The complete genome sequence of SARS-CoV-2 of accession number MT520466 was downloaded from the National Center for

Biotechnology Information (NCBI) database. The membrane glycoprotein sequence of accession number QKG90185 was selected from the complete genome and further downloaded. Antigenicity (Vaxijen v2.0 tool) and potential allergenicity (allerTop v2.1 tool) of the membrane glycoprotein sequence were evaluated using suitable online bioinformatics tools.

*B-Cell Epitope Prediction*

Linear B-cell epitopes were predicted by using the Immune Epitope Database (IEDB) with the membrane glycoprotein sequence as input. The predicted results revealed several potential B-cell epitopes, which were further tabulated in an Excel sheet to check each epitope's antigenicity and allergenicity.

*T-cell Epitope Prediction (MHC-I)*

T-cell epitopes with potential binding affinity for MHC class I molecules were predicted with the help of an antibody epitope prediction tool (IEDB) for T-cells (MHC-I). MHC-I binding epitopes were predicted and compiled in an Excel sheet, where they were filtered and sorted based on allele binding affinity. Each epitope was evaluated for allergenicity, antigenicity, toxicity, and solubility. The epitopes with the lowest $IC_{50}$ value were reported by Peters *et al.* (2020).

*T-cell Epitope Prediction (MHC-II)*

Similarly, T-cell epitopes with potential binding to MHC class II molecules were predicted by an antibody epitope prediction tool (IEDB) for T-cells (MHC-II). MHC-II binding epitopes were predicted and subsequently organized in an Excel sheet for allele-based sorting, with each epitope assessed for allergenicity, antigenicity, toxicity, and solubility. Epitopes with the lowest $IC_{50}$ values were prioritized.

*MHC class I and II Epitope File Generation*

Two files were generated from the sorted predictions for MHC-I and MHC-II binding, containing identified potential T-cell epitopes.

*Population Coverage Analysis*

Defining a minimum population coverage (e.g., 60%) for epitope selection is standard in vaccine design and immune-informatics, but the specific threshold may vary across studies. Maximum chosen HLA allele population coverage is reported to be essential in specific sources, which clarify that more coverage confers more vaccine efficacy in broader populations (Oany *et al.*, 2014). The population coverage percentages are mentioned in the results section.

*Vaccine Construction*

MHC-II, MHC-I, and B-cell epitopes were selected and conjugated to common linkers (CPGPG, EAAAK, AAY) to provide structural stability and enhance immunogenicity. A mycobacterium tuberculosis 50S ribosomal protein L7/L12 acted as an adjuvant to facilitate immune response (Khan *et al.*, 2022). A 6X histidine tag was provided for purification. The terminal construct sequence was determined and evaluated for physicochemical properties, including stability, solubility, and molecular weight.

*Adjuvant Choice and Placement*

The 50S ribosomal protein L7/L12 of *Mycobacterium tuberculosis* was chosen as an adjuvant because of its reported activity to enhance immunogenicity by activating Toll-like receptors (TLRs) and maturation of dendritic cells. The adjuvant was placed at the N-terminus of the vaccine construct to allow immune cells to recognize it early. The adjuvant was linked to the epitopes using a rigid EAAAK linker (Glu-Ala-Ala-Ala-Lys), ensuring structural separation and stability while minimizing steric hindrance.

*Linker Design and Functional Roles*

The most used linkers in multi-epitope vaccine design were chosen based on their capacity to improve epitope presentation, stability, and immune recognition. The following provides a detailed explanation of their functions and applications.

*EAAAK Linker (Glu-Ala-Ala-Ala-Lys)*

Serves as a rigid α-helical spacer to position adjuvants away from epitopes and maintains structural stability and independent domain function. It reduces steric interference between the adjuvant and epitopes.

*GPGPG Linker (Gly-Pro-Gly-Pro-Gly)*

The GPGPG linker enhances flexibility and solubility when connecting helper T-lymphocyte (HTL) epitopes for MHC-II presentation. Its glycine-proline repeats provide conformational freedom and help eliminate steric hindrance between adjacent epitopes.

*AAY Linker (Ala-Ala-Tyr)*

The AAY linker enables proteasomal cleavage for MHC-I antigen presentation of cytotoxic T-lymphocyte (CTL) epitopes while preventing junctional epitope formation and preserving the structural integrity of CTL epitopes. These linkers have been established in vaccine design against various pathogens, including SARS-CoV-2, influenza, and *Mycobacterium tuberculosis*. Linkers are very important for right epitope presentation, minimizing junctional immunogenicity, and facilitating efficient proteolytic processing.

*Epitope Assembly and Vaccine Architecture*

The multi-epitope vaccine was constructed hierarchically to boost immune recognition: The EAAAK linker connects the adjuvant (50S L7/L12) at the N-terminal to B-cell epitopes. The GPGPG linker connects the end of B-cell epitopes with the MHC I epitopes, while the AAY linker connects the end of MHC I with MHC II epitopes, followed by a 6X histidine tag. The A 6X histidine tag was incorporated at the C-terminus of the construct to facilitate nickel-based affinity chromatography during protein purification without affecting the construct`s immunogenicity or structural stability.

*Structural and Functional Validation*

The stability of the α-helical conformation of the EAAAK linker hindered protein misfolding, and the GPGPG and AAY linkers provided structural flexibility. The population coverage analysis indicated that the selected epitopes achieved about 80% to 90% global coverage, demonstrating strong binding affinity to HLA alleles (Deepthi *et al.,* 2025).

*Rationale for Design Decisions*

On top of the vaccine construct, the adjuvant facilitates early activation of the immune system via TLRs, sensitizing the immune system towards epitope recognition. Alternating AAY and GPGPG linkers optimize sproteolytic processing for CTL/HTL epitopes while maintaining conformational stability. The C-terminal His-tag placement avoided interference with N-terminal adjuvant function. This modular design allows rapid adaptation to new variants by allowing epitope replacement without altering the core adjuvant-linker framework (Yang *et al.,* 2022; Zhao *et al*., 2023)

*Safety and Immunogenicity Evaluation of the Vaccine Construct*

The vaccine construct was checked using various bioinformatics tools to confirm that it is non-toxic, non-allergenic, antigenic, and soluble. Allergenicity was assessed using the AllerTop v2 tool, antigenicity with the Vaxijen v2.0 tool, toxicity with the Toxipred tool, and solubility with the Protein-sol. ProtParam validated the stability of the vaccine construct, ensuring that it remains stable under physiological conditions (Wlodawer *et al.,* 2017; Sobolev *et al.,* 2020; Oladejo *et al.,* 2023)

*Structural Modelling and Validation*

*Secondary Structure Analysis*

PSIPRED was one of the most popular bioinformatics software programs that predicts protein secondary structure from amino acid sequence with the help of neural network-based machine learning algorithms. The input sequence classifies each residue into one of three frequent secondary structure types, such as α-helix, β-strand (sheet), or coil (Buchan *et al.,* 2024).

*Tertiary Structure Prediction & Refinement*

I-TASSER (Iterative Threading ASSEmbly Refinement) was a widely used, hierarchical method for predicting the three-dimensional (tertiary) structure of proteins from their amino acid sequences (Yang & Zhang, 2015). I-TASSER generated 3D models of the vaccine construct sequence, and the GalaxyWEB server was used to refine the 3D structure for enhanced accuracy.

*Validation of Refined Tertiary Structure*

Validation of a protein tertiary structure is important to ensure its accuracy for subsequent analyses such as function prediction, molecular docking, and dynamics studies (Zhao *et al*., 2024). The Ramachandran plot is typically generated using the PROCHECK tool. It is the most employed method for the validation of protein structure. A Ramachandran plot represents the amino acid residues' backbone dihedral angles (ψ and ϕ) in a protein structure (Andrews, 2024; Sharma *et al.,* 2025). It categorizes residues into "favored," "allowed," and "outlier" regions, based on the probability of their conformations, as calculated by steric restraints and empirical data from high-resolution protein structures. After optimizing the predicted tertiary structure using tools such as GalaxyWeb or SWISS-MODEL, the PROCHECK program is employed to generate the Ramachandran plot of the model. The model's overall quality is assessed based on the proportion of residues located in the favored and allowed regions (Poornachitra *et al*., 2023; Yurievna *et al*., 2023). A reliable model typically exhibits over 90% of residues in favored regions with few or no outliers. For example, a high-quality model usually has 90%–100% of residues in the favored areas and less than 1% in outlier regions, indicating well-defined geometry and acceptable backbone conformations (Berman *et al*., 2000).

*Molecular Docking and Dynamics*

The 3D structure of the vaccine was docked with the human TLR3 immune receptor using ClusPro to predict immune receptor interactions. The best docking model was further analysed by molecular dynamics simulation using iMODS, confirming the vaccine-receptor complex's stability and flexibility (Pisano *et al*., 2023; Shaheen *et al*., 2023).

*Codon Optimization and Insilico Cloning*

The protein sequence was back-translated to nucleotide sequence using the EMBOSS Backtranseq tool (Rice *et al.,* 2000). Codon optimization for efficient *E. coli* expression was carried out with JCat (Grote *et al.*, 2005). The pET-29a (+) vector, a bacterial expression plasmid designed for producing N-terminal S-tagged proteins with a thrombin cleavage site (Novagen, 2010), was selected for cloning. It is a plasmid DNA that can be used to clone and express genes of interest in bacteria. The "+" indicates the presence of the N-terminal S-tag/thrombin configuration, which allows efficient purification of the expressed protein. Additionally, pET-29a(+) offers an optional C-terminal His-tag for enhanced purification flexibility. The optimized gene was then inserted *in silico* into the pET-29a(+) vector using SnapGene (GSL Biotech LLC) to simulate the laboratory cloning processes (Khan *et al*., 2020; Seadawy *et al.,* 2022).

*Immune Simulation*

An in silico immune simulation was also performed using the C-ImmSim server to predict the vaccine's ability to trigger B-cell and T-cell responses and cytokine release.

## Results and Discussion

*Sequence Retrieval and Analysis of Its Antigenic and Allergenic Properties*

The SARS-CoV-2 membrane glycoprotein sequence retrieved from NCBI (https://www.ncbi.nlm.nih.gov/protein/QKG90185) was evaluated for its suitability as a vaccine target. Using VaxiJen v2.0 (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html), the protein was confirmed to be antigenic, with an antigenicity score of 0.5102 (>0.4), indicating its potential to elicit an immune response. Allergenicity analysis with AllerTOP v2.1 (https://www.ddg-pharmfac.net/allertop_test/) confirmed that the protein is non-

allergenic, suggesting it would not trigger allergic reactions in humans (Fiodorova *et al*., 2022; Ogbeide *et al*., 2022).

*B Cells & T Cells Epitope Prediction & Population Coverage Analysis*

The IEDB analysis resource (https://www.iedb.org/ ) was used to predict T-cell and B-cell epitopes and conduct population coverage analysis. The predicted epitopes were organized, filtered, and sorted in Excel for systematic analysis. Priority was given to epitopes with the lowest $IC_{50}$ values (Després *et al*., 2023; Domatskiy *et al*., 2023). The screened epitopes are further evaluated for their allergenicity, antigenicity, toxicity, and solubility. Only those epitopes that were antigenic, non-allergenic, non-toxic, soluble, and exhibited low $IC_{50}$ values were selected. This process resulted in multiple B-cell and T-cell epitopes with high population coverage (MHC-I: ~95%, MHC-II: ~80.5%), which were subsequently chosen for vaccine construction (Naveed *et al*., 2021; Rashidi *et al*., 2022; Sarma *et al*., 2022; Praveen, 2024; Yu *et al*., 2024; Deepthi *et al*., 2025).

*Vaccine Construction*

The final chimeric protein vaccine construct comprised an adjuvant, six B-cell epitopes, eight MHC-I epitopes, and six MHC-II epitopes connected with appropriate linkers, as illustrated in **Figures 1 and 2.** The constructed vaccine was validated to be antigenic (0.4320 > 0.4), non-toxic, non-allergenic, soluble (0.469 > 0.45), and stable (instability index 26.96) using the following bioinformatics tools: VaxiJen v2.0 for antigenicity prediction (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html), ToxinPred for toxicity assessment (https://webs.iiitd.edu.in/raghava/toxinpred/prot_test.php), AllerTOP v2.1 for allergenicity evaluation (https://www.ddg-pharmfac.net/allertop_test/), ProtParam for physicochemical properties and protein stability (https://web.expasy.org/protparam/), and Protein-Sol for solubility prediction (https://protein-sol.manchester.ac.uk/).

| vaccine construct | |
|---|---|
| Adjuvant | 50S ribosomal protein L7/L12 |
| linker | EAAAK |
| B cell epitope | NGTITVEELKKLLEQW |
| | AN |
| | PLLESE |
| | IKD |
| | KLGASQRVAGDS |
| | YRIGNYKLNTDHSSSSDNIA |
| linker | GPGPG |
| MHC I | ANRNRFLYI |
| | ASFRLFARTR |
| | ATSRTLSYYK |
| | GTITVEELK |
| | NGTITVEELK |
| | RTRSMWSFNP |
| | TSRTLSYYK |
| | YYKLGASQR |
| linker | AAY |
| MHC II | ACFVLAAVY |
| | ACLVGLMWL |
| | AGDSGFAAY |
| | AIAMACLVG |
| | ELVIGAVIL |
| 6X Histadine tag | HHHHHH |

**Figure 1.** Vaccine construct using adjuvant B cell epitope linkers, T cell epitope followed by a histidine tag.

MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAA
AGAAPAGAAVEAAEEQSEFDVILEAAGDKKIGVIKVVREIVSGLG
LKEAKDLVDGAPKPLLEKVAKEAADEAKAKLEAAGATVTVKEA
AAKNGTITVEELKKLLEQWEAAAKANEAAAKPLLESEEAAAKIK
DEAAAKKLGASQRVAGDSEAAAKYRIGNYKLNTDHSSSSDNIAG
PGPGANRNRFLYIGPGPGASFRLFARTRGPGPGATSRTLSYYKGPG
PGGTITVEELKGPGPGNGTITVEELKGPGPGRTRSMWSFNPGPGP
GTSRTLSYYKGPGPGYYKLGASQRAAYACFVLAAVYAAYACLVG
LMWLAAYAGDSGFAAYAAYAIAMACLVGAAYELVIGAVILHHHH
HH

**Figure 2.** Vaccine construct sequence indicates the blue-highlighted region represents the adjuvant sequence, the yellow-highlighted region represents linkers, and the non-highlighted regions are epitopes.

*Secondary Structure Analysis*

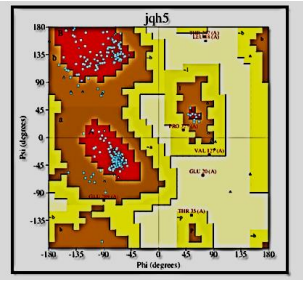
In the vaccine construct sequence, secondary structure prediction and analysis were performed using the PSIPRED tool (https://bioinf.cs.ucl.ac.uk/psipred/) to predict the 2D structure and identify the helices and coils.

*Tertiary Structure Prediction & Refinement*

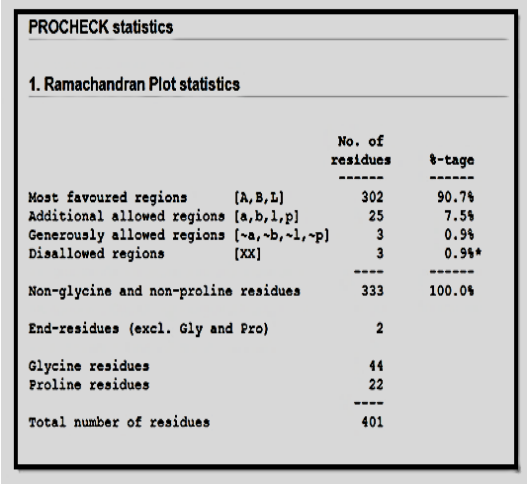
I-TASSER (https://zhanggroup.org/I-TASSER/) was used to predict the tertiary structure of the vaccine construct and generate 3D models. The resultant models were selected by C-score (confidence score), where a value greater than -1.5 indicates a reliable fold, and the TM-score assesses structural similarity to native proteins (a TM-score > 0.5 suggests correct topology) (Yang & Zhang, 2015). Hence, 3D model-1 was selected as the best, with a C-score of -0.5 (> 1.5) and a TM-score of 0.65 (>0.5), representing the highest scoring model among the predictions. The 3D structure was further refined using the GalaxyWEB server to enhance accuracy (Dorn *et al*., 2024; Marian *et al*., 2024).

*Tertiary Structure Validation*

The Ramachandran plot is an essential tool for evaluating the accuracy and reliability of a protein's 3D structure. Computational models with over 90% of residues in the most favored regions are generally considered high-quality structures, while less than 1% in disallowed regions indicates a trustworthy structure (Hooft *et al*., 1997). Ramachandran analysis of the 3D model showed that the majority of residues were located in favourable regions, as illustrated in **Figures 3a and 3b**. Structural validation using PROCHECK confirmed that 90.7% of residues resided in favored regions, reflecting a consistent, stable, and valid model. This analysis supports the suitability of the selected protein model for vaccine design (Wlodawer *et al*., 2017; Sobolev *et al.,* 2020; Oladejo *et al.,* 2023).




a)



PROCHECK statistics

1. Ramachandran Plot statistics

| | | No. of residues | %-tage |
|---|---|---|---|
| Most favoured regions | [A,B,L] | 302 | 90.7% |
| Additional allowed regions | [a,b,l,p] | 25 | 7.5% |
| Generously allowed regions | [~a,~b,~l,~p] | 3 | 0.9% |
| Disallowed regions | [XX] | 3 | 0.9%* |
| Non-glycine and non-proline residues | | 333 | 100.0% |
| End-residues (excl. Gly and Pro) | | 2 | |
| Glycine residues | | 44 | |
| Proline residues | | 22 | |
| Total number of residues | | 401 | |

b)

**Figure 3.** In the Ramachandran plot, most of the blue coloured dots that represent residues are in the red coloured favoured regions, indicating that the protein structure is predicted accurately and hence the validation is confirmed (a), and the PROCKECK analysis of the Ramachandran plot states that the most favoured regions above 90% are the stable and validated protein (b)

*Molecular Docking and Dynamics*

The vaccine construct exhibited strong binding affinity to TLR3 in the docking and simulation and formed a stable complex in molecular dynamics simulations. These stable interactions with TLR3 suggest potential for immune activation (Mubayrik *et al*., 2022).

*Codon Optimization and In-silico Cloning*

The protein sequence was reverse translated to nucleotide sequence using EMBOSS Backtranseq, and codon optimization for efficient *E. coli* expression was performed with the JCat tool. The optimized gene sequence was fragmented using SnapGene and then inserted into the pET-29a (+) vector at common restriction sites (NcoI and BstEII) for virtual cloning and plasmid construct visualization. The pET-29a (+) vector of length 5371bp and the NcoI and BstEII sites are selected as they are the common restriction sites in both the vector and the fragment. The improved sequence from JCAT is converted into a fragment of length 1203bp by the SNAPGENE tool, and the common restriction sites NcoI & BstEII in this fragment are also selected. Both the resultant vector to be cloned is of length 4277bp, and the fragment of length 1030bp is cloned with respect to their selected common restriction sites, and the resulting cloned vector of length 5307bp is shown in **Figure 4.**

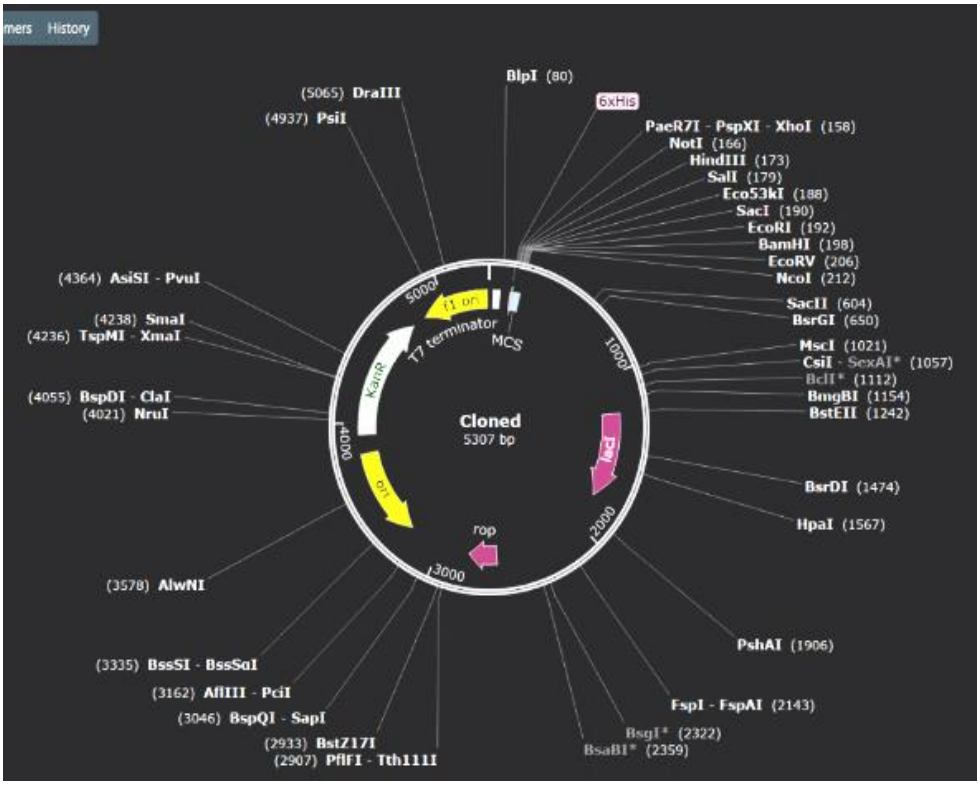



**Figure 4.** The result of the cloned vector of length 5307bp.

*Immune Simulation*

In the final step, *in-silico* immune simulations of the vaccine sequence were performed using the C-ImmSim tool to predict the immune response profile. The results demonstrated the vaccine's ability to stimulate B-cell and T-cell responses, along with cytokine production, upon administration in the human body **(Figure 5)** (AlShammasi *et al*., 2024; Ludovichetti *et al*., 2024; Menhadji *et al*., 2024).

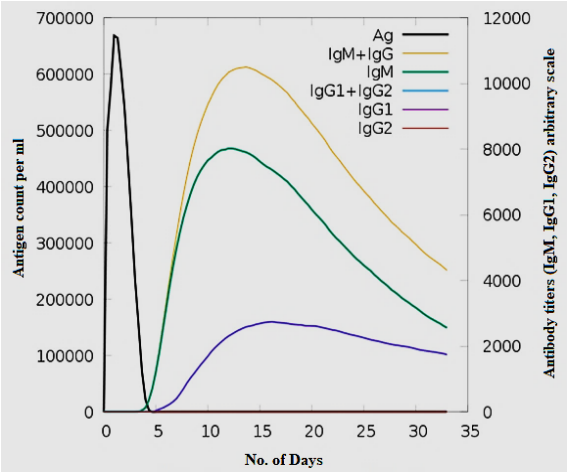



**Figure 5.** The in silico immune response generated by the C-immSim server in the human body after the vaccine is injected.

In silico prediction of the SARS-CoV-2 membrane glycoprotein (Rakib *et al.,* 2020) provided valuable insights into potential B-cell and T-cell epitopes. Initial analysis validated the protein`s antigenic and non-allergic properties, thus making it a promising candidate for vaccine development. Linear B-cell epitope prediction highlighted six regions as potential targets for antibody-mediated immune responses. For T-cell immunity, MHC-I binding epitopes were predicted and then classified based on allele specificity, enabling the identification of epitopes with high-binding affinity for various MHC-I alleles. Similarly, MHC-II binding epitope prediction and topological analysis provided a list of candidate helper T-cell epitopes (Dipalma *et al*., 2022; Zhao *et al*., 2022). Separate files for MHC-I and MHC-II epitopes were created to facilitate further selection for incorporation into a multi-epitope vaccine construct. Applying in silico software significantly simplifies the initial phase of designing vaccines. Using computational tools streamlines the early stages of vaccine design by identifying likely immunogenic regions, reducing the need for labor-intensive experimental screening. Additionally, predicting epitopes that bind multiple MHC alleles allows for designing vaccines with broad population coverage (MHC-I: 95%, and MHC-II: 80.49%), which is critical for controlling pandemic diseases worldwide (Arios-Caro *et al*., 2022; Canassa *et al*., 2022).

In constructing the vaccine, including an adjuvant and strategically designed linkers enhances both immunogenicity and structural stability, as illustrated in **Figures 1 and 2**. Structural modelling, molecular docking and simulation, codon optimization, *in silico* cloning, and immune simulation collectively support the vaccine`s potential efficacy and safety. Protein structure validation using the Ramachandran plot confirmed proper folding, indicating the distribution of residues across different regions such as the red region represents the most favored residues (90.7%), the brown region denotes additional favored residues (7.5%), the yellow region shows generously allowed residues (0.9%). The white region indicates disallowed residues (0.9%), as indicated in **Figure 3.** The *pET-29a(+)* vector was chosen for cloning due to its widespread use in expressing proteins for vaccines, including SARS-CoV-2 (Xu *et al*., 2021). Its strong T7 promoter enables high-level expression of the target protein in combination with T7 RNA polymerase. The vector also contains multiple unique restriction sites upstream of the T7 promoter, simplifying the insertion and cloning of DNA fragments. Furthermore, the lac operator and self-encoding lac repressor allow regulated expression, minimizing unintended protein production and potential host toxicity (Baneyx *et al*., 1999). The optional C-terminal His-tag facilitates protein purification and analysis (Terpe *et al*., 2003). With a medium copy number, the *pET-29a(+)* vector balances high protein yield with host cell viability, and its effectiveness is well-documented in numerous studies (Ingle *et al*., 2023; Shaheen *et al*., 2023).

## Conclusion

The study employed an *in silico* approach to predict potential B-cell and T-cell epitopes of the SARS-CoV-2 membrane glycoprotein. The predicted immunogenic epitopes, identified through bioinformatics tools for their ability to induce both humoral and cellular immune responses, form the basis for the rational design of a multi-epitope vaccine candidate. *In silico* vaccine design, leveraging advanced bioinformatics platforms, represents a promising strategy for combating SARS-CoV-2 and other emerging infectious diseases (Negreiros *et al*., 2024; Omokunle, 2024). The proposed vaccine candidate exhibits favorable immunogenic, physicochemical, and safety properties, indicating strong potential for experimental validation and future clinical application. This approach underscores the transformative impact of computational vaccinology in modern vaccine development. The use of bioinformatics tools significantly accelerates the early stages of vaccine design. However, further *in silico* analyses, such as population coverage, epitope conservancy, and immune simulation, followed by experimental validation, are essential to fully establish the efficacy and potential of the designed multi-epitope vaccine (Anushree *et al*., 2023; Mollah *et al*., 2023).

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**Ethics statement:** None

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