

EFFECT OF KOZAK AND SIGNAL PEPTIDE SEQUENCES ON RECOMBINANT ANTIBODY EXPRESSION IN CHO CELLS TARGETING OPISTHORCHIS VIVERRINI ANTIGEN

Piyawadee PONGPHAN^{1,4}, Watcharin LOILOME^{1,4}, Nisana NAMWAT^{1,4}, Arporn WANGWIWATSIN^{1,4}, Attapol TITAPUN^{3,4}, Paiboon SITHITHAWORN^{2,4} and Poramate KLANRIT^{1,4*}

1 Department of Systems Biosciences and Computational Medicine, Faculty of Medicine, Khon Kaen University, Thailand; porakl@kku.ac.th (Corresponding Author)

2 Department of Parasitology, Faculty of Medicine, Khon Kaen University, Thailand

3 Department of Surgery, Faculty of Medicine, Khon Kaen University, Thailand

4 Cholangiocarcinoma Research Institute, Khon Kaen University, Thailand

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ABSTRACT

Opisthorchis viverrini (OV) infection is a major public health concern in Southeast Asia and is strongly associated with cholangiocarcinoma. Reliable production of monoclonal antibodies is essential for antigen-based diagnostic assays; however, conventional hybridoma systems may have limitations in stability and scalability. This study aimed to develop and optimize a recombinant antibody expression system targeting OV antigen using mammalian cells. Variable heavy-chain (VH) and light-chain (VL) sequences derived from the OV-03 hybridoma clone were cloned into the pTRIOZ-hIgG1 vector to generate two constructs: (+Kozak, +SP) and (−Kozak, −SP). Following transfection into CHO-K1 cells, gene expression was confirmed by PCR, demonstrating successful transcription in both constructs. However, Western blot analysis revealed intracellular expression of IgG heavy chain (~50 kDa), light chain (~28 kDa), and full-length antibody (~150 kDa) only in cells transfected with the (+Kozak, +SP) construct, highlighting the essential roles of the Kozak sequence and signal peptide in efficient protein expression. Additionally, increasing fetal bovine serum (FBS) concentration (1.25-10%) enhanced intracellular antibody levels. These findings demonstrate that optimized translational elements and culture conditions significantly improve recombinant antibody production. This study provides a foundation for scalable antibody production and supports the development of recombinant antibodies for diagnostic applications in opisthorchiasis.

Keywords: Recombinant Antibody, *Opisthorchis viverrini*, CHO-K1 cells

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INTRODUCTION

Opisthorchiasis is endemic in Southeast Asia, particularly in Thailand. *Opisthorchis viverrini* is a liver fluke that infects humans through the consumption of raw or undercooked fish and has a high prevalence in the northeastern region of Thailand. It has been classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC) and is directly associated with the development of cholangiocarcinoma (Sripa et al., 2012; Srithai et al., 2021). There are diagnostic tools for the detection of *O. viverrini* infection in various sample types, such as the detection of OV eggs in fecal samples (Charoensuk et al., 2019), OV-specific antibodies in blood samples (Sadaow et al., 2022), and OV antigens in fecal or urine samples (Worasith et al., 2019). Currently, OV antigen test kits are available for detecting *O. viverrini* antigens in urine samples (Worasith et al., 2015). These tests are rapid, accurate, and suitable for field screening. The kits are based on the antigen-antibody principle, in which antibodies are traditionally produced using a hybridoma system. In this method, mice are immunized with OV antigen, and B cells capable of producing specific antibodies are collected and fused with myeloma cells to generate immortalized cell lines that continuously produce antibodies. However, this system has stability issues due to mutations and chromosomal loss, which can affect cDNA integrity and lead to variability in antibody production between batches (Siripanthong et al., 2021). For this reason, recombinant DNA technology is adopted to replace these systems, as it provides greater consistency and reduces the risks associated with hybridoma-based antibody production. However, the expression of recombinant proteins in mammalian cells is complex and requires optimization, as post-translational modifications occur. Therefore, this study aims to compare and optimize the effects of the Kozak sequence and signal peptide on the expression of recombinant antibodies against the OV antigen in Chinese Hamster Ovary (CHO) cells, and to investigate the effect of fetal bovine serum (FBS) concentration on intracellular antibody production.

LITERATURE REVIEWS

Cholangiocarcinoma and risk factors of CCA

Cholangiocarcinoma (CCA) is a malignant neoplasm of the bile duct epithelium, classified into intrahepatic (iCCA) and extrahepatic (eCCA) forms, including perihilar and distal subtypes. Tumor location critically influences diagnosis, therapeutic strategies, and prognosis. CCA is associated with poor clinical outcomes due to the lack of early symptoms, leading to delayed diagnosis. The incidence is particularly high in Southeast Asia, especially in Thailand, where environmental and infectious risk factors are prevalent. (Banales et al., 2016). Major risk factors include chronic biliary inflammation, structural abnormalities, and exposure to carcinogenic compounds (Gupta & Dixon, 2017), and parasitic infections. Diseases such as bile duct cysts, gallstones, and primary sclerosing cholangitis contribute to sustained epithelial injury and increased malignant transformation (Nordenstedt et al., 2012; Ilyas & Gores, 2013). Notably, infection with the liver fluke *O. viverrini* represents a major etiological factor in endemic regions and is associated with the consumption of raw or undercooked freshwater fish (Sripa et al., 2011).

The pathogenesis of CCA of parasitic infection involves mechanical damage to the bile duct epithelium (Liau et al., 2023), chronic inflammation, and the action of parasite-derived excretory-secretory products. These processes activate inflammatory signaling pathways, such as nuclear factor- κ B, leading to increased oxidative stress, nitric oxide production, DNA damage, and genomic instability. Repeated cycles of injury and repair result in epithelial hyperplasia, fibrosis, and structural alterations, thereby promoting cholangiocarcinogenesis. (Sripa & Pairojkul, 2008).

Current diagnostic methods for *O. viverrini* infection and monoclonal antibody production

Current diagnostic methods for infection with *O. viverrini* include parasitological, molecular, serological, and antigen-based approaches. Stool examination techniques, such as Kato-Katz and formalin-ethyl acetate concentration (FECT), are widely used due to their simplicity and low cost, although their sensitivity is limited in low-intensity infections (Charoensuk et al., 2019).

Molecular methods, particularly polymerase chain reaction (PCR), provide high sensitivity and specificity but require specialized laboratory infrastructure (Lovis et al., 2009; Worasith et al., 2019).

Serological assays such as enzyme-linked immunosorbent assay (ELISA) detect antibodies against parasite antigens and are useful for screening, although they cannot distinguish between past and current infection (Sadaow et al., 2022).

Urine-based antigen detection has recently emerged as a non-invasive method with improved diagnostic performance. These advancements highlight the ongoing effort to develop diagnostic tools that are accurate, rapid, and practical for use in endemic regions (Worasith et al., 2015).

Monoclonal antibodies (mAbs) are critical tools in diagnostic development. While hybridoma technology enables the production of highly specific antibodies, it is often time-consuming and resource-intensive (Mitra & Tomar, 2021).

In contrast, recombinant DNA technology allows efficient, scalable antibody production and facilitates molecular engineering to enhance diagnostic sensitivity and specificity for OV antigen detection (Giudicelli et al., 2004; Müller-Sienerth et al., 2014).

Effect of Kozak and signal peptide sequences in the mammalian expression system

In mammalian expression systems, the Kozak sequence critically influences translation initiation by facilitating efficient ribosomal recognition of the start codon, leading to higher recombinant protein yields. Studies demonstrate that adding Kozak upstream of target genes in CHO cells enhances transient and stable expression; for instance, Kozak alone boosts eGFP fluorescence 1.26-fold, while Kozak combined with a signal peptide sequence achieves up to 2.2-fold increases (Rita Costa et al., 2010).

Signal peptides guide nascent proteins into the ER for secretion, with optimal selection yielding up to fourfold higher expression levels by improving translocation and processing. Reviews emphasize that proper signal peptide choice, alongside Kozak optimization, dramatically impacts monoclonal antibody production in engineered cell lines (Zhang et al., 2024).

RESEARCH METHODOLOGY

Chemicals, Reagents, and Cell Sources

All chemicals and reagents used in this study were of analytical grade. For bacterial culture, Luria-Bertani (LB) broth (Thermo Fisher Scientific, USA) and zeocin (InvivoGen, USA) were used for plasmid amplification and selection. Mammalian cell culture was performed using Ham's F-12K medium (ATCC, USA) supplemented with heat-inactivated fetal bovine serum (FBS) (Himedia, USA), penicillin-streptomycin (Thermo Fisher Scientific, USA), and zeocin (InvivoGen, USA). Phosphate-buffered saline (PBS; 1X, pH 7.4) was obtained from Thermo Fisher Scientific (USA). jetOPTIMUS® DNA transfection reagent (Sartorius, Germany) was used for plasmid transfection into mammalian cells.

DNA and RNA purification were carried out using QIAprep Spin Miniprep Kit, DNeasy Blood and Tissue Kit, and RNeasy Mini Kit (Qiagen, Germany). DNA concentration was measured using the Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific, USA). The Rapid Barcoding Kit (SQK-RBK114.24) from Oxford Nanopore Technologies (UK) was used for sequencing preparation. For cDNA synthesis and polymerase chain reaction (PCR),

SuperScript™ VILO™ cDNA Synthesis Kit (Thermo Fisher Scientific, USA) and 2X Taq Master Mix (Vivantis, Malaysia) were used.

Western blot analysis was performed using protease inhibitor cocktail (Roche, Switzerland), goat anti-human IgG (H+L) secondary antibody conjugated with horseradish peroxidase (HRP) (Invitrogen, USA), enhanced chemiluminescence (ECL) substrate (GE Healthcare, UK), skim milk (Sigma, USA), and Tween-20 (Fermentas, USA).

Chinese hamster ovary cells (CHO-K1; ATCC, Cat. No. CCL-16) were obtained from the American Type Culture Collection and were used as the mammalian host cell line for recombinant antibody expression. Hybridoma cells (clone OV-03) were kindly provided by Professor Dr. Paiboon Sithithaworn for antibody gene isolation. The mammalian expression vector pTRIOZ-hIgG1 (InvivoGen, USA) was used for high-level expression of full-length recombinant human IgG antibodies.

Plasmid engineering and optimization

The plasmid construct encoding the antibody sequence was kindly provided by Professor Dr. Paiboon Sithithaworn. The plasmid contained the variable regions of the heavy chain (VH) and light chain (VL) specific to *O. viverrini* antigen but did not include Kozak or signal peptide sequences (–Kozak, –SP). For the optimized construct, the plasmid was sent to a company to incorporate Kozak and signal peptide sequences upstream of the VH and VL regions (+Kozak, +SP) in order to compare the expression efficiency between the two plasmid constructs.

Plasmid Isolation

After inoculating *E. coli* JM108 in the flask, plasmid DNA was isolated using the QIAprep Spin Miniprep Kit (QIAGEN, Germany). The isolated plasmid was then transfected into CHO-K1 cells.

Plasmid transfection into CHO-K1 cells

CHO-K1 cells were seeded at a density of 50,000 cells per well in a 24-well plate (Corning, USA) and incubated in a 5% CO₂ incubator at 37 °C for 24 hours. Subsequently, 0.5 µg of each DNA plasmid construct (+Kozak, +SP) and (–Kozak, –SP) was diluted in 50 µL of jetOPTIMUS® buffer, and 0.5 µL of jetOPTIMUS® transfection reagent was added to the DNA solution. The transfection mixture was incubated at room temperature for 10 minutes. After incubation, 50 µL of the mixture was added to each well. The plates were gently rocked to ensure even distribution and then incubated at 37 °C for 24 hours.

CHO-K1 cell culture

Non-transfected CHO-K1 cells were cultured in Ham's F-12K medium (ATCC, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Himedia, USA) and 1% penicillin-streptomycin (Thermo Fisher Scientific, USA). CHO-K1 cells transfected with the plasmid construct containing Kozak and signal peptide sequences (+Kozak, +SP), as well as CHO-K1 cells transfected with the plasmid construct lacking Kozak and signal peptide sequences (–Kozak, –SP), were cultured in Ham's F-12K medium (ATCC, USA) supplemented with 10% heat-inactivated FBS (Himedia, USA), 1000 µg/mL zeocin (InvivoGen, USA), and 1% penicillin-streptomycin (Thermo Fisher Scientific, USA). The cells were subcultured every three to four days in a CO₂ incubator at 37 °C.

To evaluate the effect of fetal bovine serum (FBS) concentration on intracellular antibody production, CHO-K1 cells transfected with the plasmid construct containing Kozak and signal peptide sequences (+Kozak, +SP) were cultured in medium supplemented with 1.25%, 2.5%, 5%, and 10% FBS.

RNA Extraction from CHO-K1 Cells Transfected with Two Plasmid Constructs

Three days after plasmid transfection, CHO-K1 cells were collected by centrifugation to obtain cell pellets. Total RNA was extracted using the RNeasy Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. The RNA concentration from CHO-K1 cells

transfected with each plasmid construct (+Kozak, +SP) and (-Kozak, -SP) was measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA).

Plasmid and gene expression analysis by polymerase chain reaction (PCR)

The SuperScript™ VILO™ cDNA Synthesis Kit (Thermo Fisher Scientific, USA) was used to reverse-transcribe total RNA extracted from CHO-K1 cells into complementary DNA (cDNA). The synthesized cDNA was then amplified by polymerase chain reaction (PCR) under the following cycling conditions: initial denaturation at 94 °C for 2 minutes; 40 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 30 seconds; followed by a final extension at 72 °C for 7 minutes. Specific primers targeting the VH and VL regions were used for amplification.

Table 1 Oligonucleotide primers used for amplification of immunoglobulin heavy-chain (VH) and light-chain (VL) variable regions

Primer	Sequence (5'-3')	Length (bp)	Product size
VH	Forward-CAGGTGCACCTGAAGCAGTCAGG	23	354 bp
	Reverse-TGAGGAGACGGTGACTGAGGTTC	23	
VL	Forward-CAAATTGTTCTCACCCAGTCTCC	23	318 bp
	Reverse-TTTCAGCTCCAGCTTGGTC	19	

Visualization of PCR Products using the TapeStation system

A volume of 1 µL of each PCR product and D1000 ladder was mixed with 3 µL of D1000 sample buffer. After brief centrifugation (spin down), the mixture was vortexed for 1 minute at 2,000 rpm using an IKA vortex mixer with an appropriate adapter. The samples were then loaded onto the 4200 TapeStation instrument for analysis.

Antibody Expression analysis by western blotting

Protein samples obtained from CHO-K1 cells transfected with each DNA plasmid construct (+Kozak, +SP) and (-Kozak, -SP), as well as human serum used as a positive IgG control, were quantified using a bicinchoninic acid (BCA) protein assay. A total of 30 µg of protein from each sample was separated by size using 12% SDS-PAGE under reducing conditions and 10% under non-reducing condition. In addition, protein samples from CHO-K1 cells transfected with the construct containing Kozak and signal peptide sequences (+Kozak, +SP) and cultured in media supplemented with 1.25%, 2.5%, 5%, and 10% FBS were analyzed on a separate gel to evaluate the effect of FBS concentration on antibody expression.

After electrophoresis, proteins were transferred onto a nitrocellulose membrane and blocked with 5% skim milk in Tris-buffered saline (TBS) for 1 hour at room temperature. The membrane was then incubated with goat anti-human IgG (H+L) horseradish peroxidase (HRP)-conjugated secondary antibody diluted 1:5000 (Invitrogen, USA) for 1 hour at room temperature. The membrane was washed once with TBS for 5 minutes, followed by three washes with TBS containing Tween-20 (TBS-T) for 5 minutes each. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection system (GE Healthcare, UK). The molecular weight of the recombinant antibody was determined by comparison with a protein molecular weight marker (Cat. No. ab116027, Abcam, UK).

RESEARCH RESULTS

Engineering of antibody sequence to pTRIOZ-hIgG1 plasmid

For the plasmid construct lacking Kozak and signal peptide sequences (-Kozak, -SP), the variable regions of the heavy chain (VH) and light chain (VL) specific to *O. viverrini* antigen were inserted into the pTRIOZ-hIgG1 plasmid. Restriction enzyme recognition sites were incorporated at both ends of the antibody sequences to facilitate cloning. For the VH region,

AgeI (ACCGGT) was introduced at the 5' end and NheI (GCTAGC) at the 3' end. For the VL region, SgrAI (CACCGGCG) and BsiWI (CGTACG) restriction sites were added at the 5' and 3' ends, respectively.

For the optimized plasmid construct containing Kozak and signal peptide sequences (+Kozak, +SP), a Kozak consensus sequence (CCACCATGG) (Blanco et al., 2020) was introduced upstream of both VH and VL regions to enhance translational efficiency. In addition, signal peptide sequences were incorporated at the N-terminus of both antibody chains to promote protein secretion in mammalian cells. The heavy-chain signal peptide sequence was 5'-ATGG AGTTTGGGCTGAGCTGGGTTTTCTCGTTGCTCTTTTATAGAGGTGTCCAGTGT -3', while the light-chain signal peptide sequence was 5'-ATGGACATGAGGGTCCCTGCTCAGCTCCTGGGGCTCCTGCTGCTCTGGCTCTCAGGTGCCAGATGT -3'. Restriction enzyme sites were maintained to ensure compatibility with the pTRIOZ-hIgG1 expression vector.

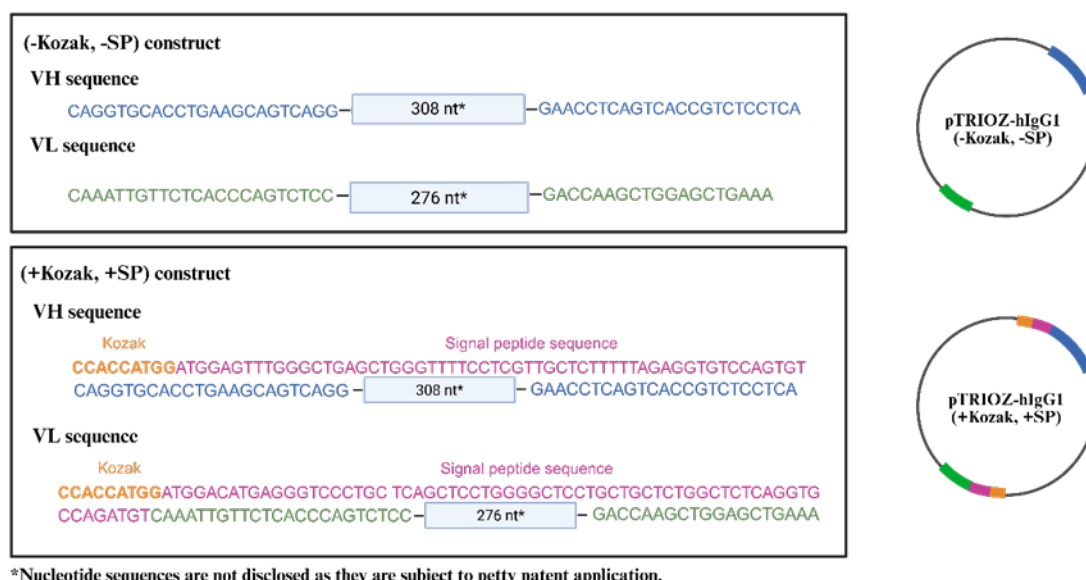


Figure 1 Schematic diagram of recombinant plasmid constructs (-Kozak, -SP) and (+Kozak, +SP) encoding VH and VL regions specific to *Opisthorchis viverrini*. Full nucleotide sequences are not shown due to a petty patent on the registration process.

mRNA expression from CHO cells transfected with plasmid construct

The RNA concentrations obtained from CHO cells transfected with plasmid constructs (+Kozak, +SP) and (-Kozak, -SP) were 284.0 ng/μL and 109.8 ng/μL, respectively.

DNA amplification was performed by polymerase chain reaction (PCR) using VH and VL primers with cDNA templates synthesized from RNA extracted from CHO-K1 cells transfected with each plasmid construct. PCR reactions were carried out using Vivantis 2X Taq Master Mix to evaluate primer specificity across samples. PCR was performed using both VH and VL primer pairs for each sample. The resulting PCR products were analyzed to evaluate gene transcription levels (mRNA expression) using an automated electrophoresis system (TapeStation).

The results showed successful PCR amplification of the target regions (inserted VH and VL sequences) in cDNA synthesized from mRNA extracted from CHO-K1 cells transfected with plasmid constructs (+Kozak, +SP) and (-Kozak, -SP). In contrast, non-transfected CHO-K1 cells showed no PCR amplification. These findings confirm that both plasmid constructs were successfully delivered into CHO-K1 cells and expressed at the mRNA level.

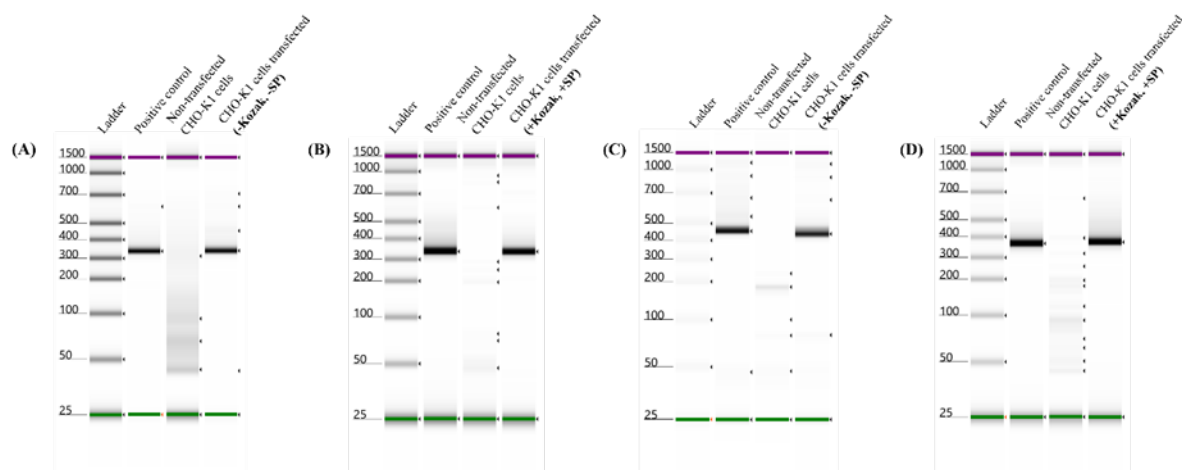


Figure 2 Gel electrophoresis analysis of PCR products obtained from purified plasmid (positive control), non-transfected CHO-K1 cells (negative control), and CHO-K1 cells transfected with plasmid constructs (-Kozak, -SP) and (+Kozak, +SP). PCR amplification was performed using VL primers for (A) plasmid construct (-Kozak, -SP) and (B) plasmid construct (+Kozak, +SP), and VH primers for (C) plasmid construct (-Kozak, -SP) and (D) plasmid construct (+Kozak, +SP)

Antibody expression of engineered pTRIOZ-hIgG1 in CHO-K1 cells by Western blot

To examine recombinant antibody expression, Western blot analysis was performed. Proteins were separated by SDS-PAGE under denaturing conditions, and antibody detection was carried out using a goat anti-human IgG secondary antibody. The samples included human serum (positive control containing human IgG), non-transfected CHO-K1 cells (negative control), and CHO-K1 cells transfected with plasmid constructs (+Kozak, +SP) and (-Kozak, -SP). The heavy and light chains of IgG were detected at approximately 50 kDa and 28 kDa, respectively. In CHO-K1 cells transfected with the (+Kozak, +SP) construct, clear bands corresponding to both chains were observed. Under non-reducing conditions, the intact recombinant IgG (~150 kDa) was detected, indicating successful antibody expression. In contrast, no detectable bands were observed in cells transfected with the (-Kozak, -SP) construct, as shown in Figure 3.

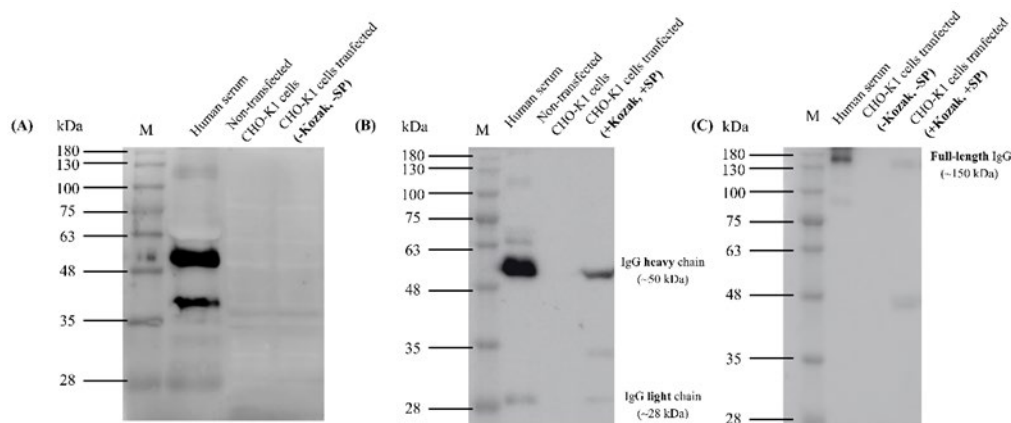


Figure 3 Western blot analysis of intracellular recombinant IgG in CHO-K1 cells transfected with plasmid constructs (A) (-Kozak, -SP) and (B) (+Kozak, +SP). Under reducing conditions, the heavy chain (~50 kDa) and light chain (~28 kDa) were clearly detected. Under non-reducing conditions (C), the full-length recombinant antibody IgG was observed at approximately 150 kDa.

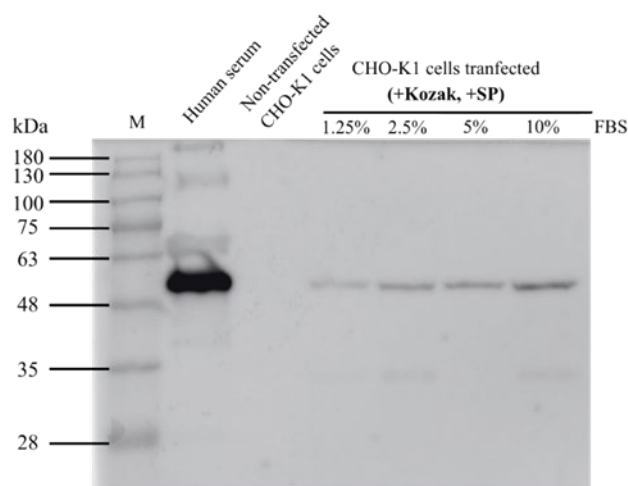


Figure 4 Western blot analysis of intracellular recombinant IgG in CHO-K1 cells transfected with plasmid construct (+Kozak, +SP) cultured under different fetal bovine serum (FBS) concentrations (1.25-10%).

DISCUSSION & CONCLUSION

This study demonstrates the successful engineering and expression of a recombinant monoclonal antibody derived from the OV-03 hybridoma clone (Worasith et al., 2023) using a mammalian expression system. The heavy- and light-chain variable sequences were successfully identified, cloned into the pTRIOZ-hIgG1 plasmid, and transfected into CHO-K1 cells, confirming the feasibility of producing recombinant antibodies for diagnostic applications. PCR amplification confirmed successful gene transfer and transcription of both plasmid constructs (+Kozak, +SP) and (−Kozak, −SP), while no amplification was observed in non-transfected cells.

Although both constructs showed detectable mRNA levels, Western blot analysis revealed that intracellular IgG heavy chain (~50 kDa) was detected only in CHO-K1 cells transfected with the (+Kozak, +SP) construct. This observation indicates that the incorporation of the Kozak consensus sequence and signal peptide is required for intracellular protein production in our system. The Kozak sequence is known to improve ribosomal recognition of the start codon, while signal peptides facilitate targeting of nascent polypeptides to the endoplasmic reticulum, supporting proper folding and processing of recombinant proteins. The absence of detectable protein in the (−Kozak, −SP) construct, despite measurable mRNA expression, highlights the importance of optimized sequence elements for effective recombinant antibody production (Blanco et al., 2020; Haryadi et al., 2015).

The antibody produced in this study was detected primarily in intracellular fractions, indicating successful translation and suggesting proper assembly, as demonstrated by the presence of the heavy chain (50 kDa), light chain (28 kDa) and full-assembled structure (150 kDa) of recombinant antibody. However, the secretion of antibodies is also important for harvesting in large-scale production. The next goal of our research will be to detect secreted antibodies in the culture medium to confirm the secretion pathway. To our assumption, the antibody will be secreted in the culture medium as presented in Chinakrapong et al (Chinakrapong et al., 2025). Finally, the harvested antibody from culture medium will be tested the activity to OV-antigen via ELISA.

Fetal bovine serum (FBS) provides essential nutrients and growth factors that support cellular metabolism, which may contribute to enhanced recombinant protein yield (Lee et al., 2022). Culture conditions (% FBS) also influenced recombinant protein expression. Increasing FBS concentration from 1.25% to 10% resulted in increased intracellular antibody, suggesting that

higher serum concentrations promote cell growth and protein synthesis in an adherent culture system. However, to increase recombinant antibody production on a large scale in the future, CHO cells will be adapted to lower % FBS or to use a chemically defined culture medium (FBS-free) to obtain suspended cells, and then cultured in a suspension cell bioreactor. (Miki & Takagi, 2015). Using chemically-defined culture medium will reduce FBS lot variability but enhance batch-to-batch production consistency, which is preferred in industrial processes (Brunmaier & Walker, 2025).

In conclusion, this study successfully demonstrates the production of a recombinant monoclonal antibody against the *O. viverrini* antigen by cloning VH and VL sequences into the pTRIOZ-hIgG1 expression vector and expressing it in CHO-K1 cells. The results indicate that incorporation of the Kozak sequence and signal peptide significantly improves intracellular antibody expression, confirming the importance of optimized translational elements in mammalian expression systems. These findings confirm the feasibility of producing recombinant antibodies targeting *O. viverrini* in CHO-K1 cells and provide a basis for further studies on antibody secretion and adaptation to low-FBS culture for large-scale production. The recombinant antibody developed in this study has potential applications for improving the consistency and scalability of antibody production for opisthorchiasis diagnostic assays.

REFERENCES

- Brunmaier, L. A. E., & Walker, T. W. (2025). Chemically-defined medium formulation and adaptation method for supporting growth of endothelial cells. *Scientific Reports*, 15(1), 35458.
- Charoensuk, L., Subrungruang, I., Mungthin, M., Pinlaor, S., & Suwannahitatorn, P. (2019). Comparison of stool examination techniques to detect *Opisthorchis viverrini* in low intensity infection. *Acta Tropica*, 191, 13-16.
- Chinakarapong, M., Kotemul, K., Hanprathet, S., Thu, M. M., Pata, S., Laopajon, W., Kasinrer, W., & Takheaw, N. (2025). Intracellular Antibody Levels Predict Secretion Efficiency in HEK293T Cells Expressing a Chimeric Anti-CD99 Antibody from a Single-Expression Plasmid. *Biotechnology Journal*, 20(10), e70129.
- Lee, D. Y., Lee, S. Y., Yun, S. H., Jeong, J. W., Kim, J. H., Kim, H. W., Choi, J. S., Kim, G.-D., Joo, S. T., Choi, I., & Hur, S. J. (2022). Review of the Current Research on Fetal Bovine Serum and the Development of Cultured Meat. *Food Science of Animal Resources*, 42(5), 775-799.
- Miki, H., & Takagi, M. (2015). Design of serum-free medium for suspension culture of CHO cells on the basis of general commercial media. *Cytotechnology*, 67(4), 689-697.
- Rita Costa, A., Elisa Rodrigues, M., Henriques, M., Azeredo, J., & Oliveira, R. (2010). Guidelines to cell engineering for monoclonal antibody production. *European Journal of Pharmaceutics and Biopharmaceutics*, 74(2), 127-138.
- Sadaow, L., Rodpai, R., Janwan, P., Boonroumkaew, P., Sanpool, O., Thanchomnang, T., Yamasaki, H., Ittiprasert, W., Mann, V. H., Brindley, P. J., Maleewong, W., & Intapan, P. M. (2022). An Innovative Test for the Rapid Detection of Specific IgG Antibodies in Human Whole-Blood for the Diagnosis of *Opisthorchis viverrini* Infection. *Tropical Medicine and Infectious Disease*, 7(10), 308.
- Siripanthong, S., Techasen, A., Nantasenamat, C., Malik, A. A., Sithithaworn, P., Leelayuwat, C., & Jumnainsong, A. (2021). Production and characterization of antibody against *Opisthorchis viverrini* via phage display and molecular simulation. *PLOS ONE*, 16(3), e0248887.
- Sripa, B., Brindley, P. J., Mulvenna, J., Laha, T., Smout, M. J., Mairiang, E., Bethony, J. M., & Loukas, A. (2012). The tumorigenic liver fluke *Opisthorchis viverrini*-multiple pathways to cancer. *Trends in Parasitology*, 28(10), 395-407.

- Srithai, C., Chuangchaiya, S., Jaichuang, S., & Idris, Z. M. (2021). Prevalence of *Opisthorchis viverrini* and its Associated Risk Factors in the Phon Sawan District of Nakhon Phanom Province, Thailand. *Iranian Journal of Parasitology*.
- Worasith, C., Kamamia, C., Yakovleva, A., Duenngai, K., Wangboon, C., Sithithaworn, J., Watwiengkam, N., Namwat, N., Techasen, A., Loilome, W., Yongvanit, P., Loukas, A., Sithithaworn, P., & Bethony, J. M. (2015). Advances in the Diagnosis of Human Opisthorchiasis: Development of *Opisthorchis viverrini* Antigen Detection in Urine. *PLOS Neglected Tropical Diseases*, 9(10), e0004157.
- Worasith, C., Sithithaworn, J., Wongphutorn, P., Homwong, C., Khongsukwiwat, K., Techasen, A., Kopolrat, K. Y., Loilome, W., Namwat, N., Thinkamrop, B., Tawarungruang, C., Titapun, A., Laha, T., Andrews, R. H., Taylor-Robinson, S. D., & Sithithaworn, P. (2023). Accuracy of a new rapid diagnostic test for urinary antigen detection and assessment of drug treatment in opisthorchiasis. *Infectious Diseases of Poverty*, 12(1), 102.
- Worasith, C., Wangboon, C., Duenngai, K., Kiatsopit, N., Kopolrat, K., Techasen, A., Sithithaworn, J., Khuntikeo, N., Loilome, W., Namwat, N., Yongvanit, P., Carlton, E. J., & Sithithaworn, P. (2019). Comparing the performance of urine and copro-antigen detection in evaluating *Opisthorchis viverrini* infection in communities with different transmission levels in Northeast Thailand. *PLOS Neglected Tropical Diseases*, 13(2), e0007186.
- Zhang, J., Du, C., Pan, Y., Zhang, Z., Feng, R., Ma, M., & Wang, T. (2024). Optimization of a novel expression system for recombinant protein production in CHO cells. *Scientific Reports*, 14(1), 24913.

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