

DOI 10.24425/pjvs.2026.158508

Original article

# Optimized high-yield expression and antigenic characterization of a soluble pseudorabies virus gE protein in *E. coli*

Y.B. Wang, S.H. Wang, Y.C. Gao, J.W. Feng, F.Q. Zhang

School of Public Health, Henan Medical University, Xinxiang, Henan, China

Correspondence to: Y.B. Wang, e-mail: wangyinbiao118@163.com

## Abstract

Pseudorabies (PR) is an infectious disease caused by the pseudorabies virus (PRV), which can infect multiple animal species and humans. Glycoprotein E (gE) of PRV plays a vital role in viral neuroinvasion but is not essential for viral replication, making it a key target for differentiation between infected and vaccinated animals. For such serological monitoring, a large amount of purified and bioactive gE protein is essential as a diagnostic antigen. While *Escherichia coli* (*E. coli*) is an attractive system for its high yield, low cost, and rapid production, the expression of recombinant gE in this prokaryotic host is frequently hampered by protein accumulation into insoluble inclusion bodies, which compromises the native protein structure and diminishes its antigenic activity for reliable diagnostic use. The objective of this study was to develop a robust strategy for the efficient production of water-soluble gE in *E. coli* to obtain a functionally active antigen for diagnostic applications. The solubility of recombinant gE protein was optimized using a combination of low induction temperature, reduced isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) concentration, and prolonged expression time to favor proper protein folding. Through systematic optimization, we established that induction at 25°C with 0.4 mM IPTG for 8 h enabled the high-yield expression of water-soluble gE in *E. coli*, yielding approximately 35 mg/L of purified protein. Crucially, the water-soluble gE protein retained native epitopes, as demonstrated by its strong immunoreactivity with clinical PRV-positive swine sera and the ability of gE-specific antisera to recognize wild-type PRV in infected cells. This study provides a practically significant solution for producing a high-fidelity gE antigen and the cultivation-based optimization strategy presents a universal framework for solving the persistent challenge of protein aggregation into insoluble inclusion bodies.

**Keywords:** pseudorabies virus, optimization of expression conditions, water-soluble gE protein, antigenicity characterization



## Introduction

Pseudorabies (PR), also known as Aujeszky's disease, is a contagious disease caused by pseudorabies virus (PRV) which can infect multiple species, including humans (Mettenleiter 2000a, Mettenleiter 2020b). The PRV, also known as Suid herpesvirus 1, belongs to the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, and genus *Varicellovirus* (Pomeranz et al. 2005). Domestic and wild swine serve as its natural hosts, while various wild animals and domestic species act as non-natural hosts (Mettenleiter 2000a, Tischer and Osterrieder 2010). Since 2017, PRV has been confirmed to cross species barriers and infect humans, causing endophthalmitis (Ai et al. 2018, Ou et al. 2020). Subsequent case reports have further demonstrated its ability to induce severe encephalitis in humans (Hou et al. 2022, Liu et al. 2022).

The genome of PRV is a linear double-stranded DNA molecule of approximately 150 kb in size and encodes 11 glycoproteins that function at different stages of the viral replication cycle (Bo and Li 2022, Pomeranz et al. 2005). The glycoprotein E (gE) of PRV plays a critical role in viral neuroinvasion but is non-essential for viral replication (Bude et al. 2024, Polcicova et al. 2005). As a result, gE gene-deleted vaccines have been widely adopted as marker vaccines in PRV eradication programs to differentiate wild-type virus infection from vaccine immunization (Ma et al. 2024, Wang et al. 2019). The gE protein is a type I transmembrane protein, structurally divided into an N-terminal signal peptide, an extracellular domain, a transmembrane domain, and an intracellular domain (Polcicova et al. 2005). To allow the detection and surveillance of antibodies against PRV, a large amount of purified and bioactive gE protein is highly needed. While the recombinant gE protein has been expressed in diverse systems such as *Escherichia coli* (*E. coli*), yeast, and insect cells, its production is frequently hampered by issues including high cost, extended preparation periods, non-secretory expression, and protein aggregation (Serena et al. 2013, Wu et al. 2017, Yong et al. 2005). Notably, *E. coli*-based expression offers relative advantages in yield, cost, and production speed. However, a major drawback is the frequent accumulation of recombinant proteins as insoluble inclusion bodies, which can significantly compromise the native protein structure and the antigenic activity (Rosano and Ceccarelli 2014, Wingfield et al. 2014).

There are several strategies that can be used to increase the proper folding of target proteins: (1) co-expression with other genes, such as chaperones, (2) using the vector with a large protein tag, and (3) changing the culture conditions of the host cells (Eskandari et al.

2024, Gopal and Kumar 2013). However, the co-expression of molecular chaperones introduces additional complexity and undesired uncertainty into the genetic engineering process (Martínez-Alonso et al. 2007). The application of large fusion tags may affect the protein's native activity and its subsequent applications (Costa et al. 2013). Optimization of culture conditions emerges as a relatively superior strategy, as it is a non-genetic approach that is cost-effective, readily scalable, and offers distinct advantages for industrial manufacturing. For application-oriented proteins such as gE, optimizing culture conditions is a highly practical and efficient approach. However, systematic studies on the optimization of culture conditions for the expression of PRV gE protein remain relatively unexplored.

Based on the above background, this study aims to achieve high-level expression of water-soluble gE protein in *E. coli* by systematically optimizing key cultivation parameters, including induction temperature, inducer concentration, and induction duration. The expressed gE protein was then purified using Ni-NTA chromatography and evaluated for its antigenicity by enzyme-linked immunosorbent assay (ELISA) and immunoperoxidase monolayer assay (IPMA) to assess its potential as a diagnostic antigen. This study provides a reliable solution for the large-scale and cost-effective production of water-soluble PRV diagnostic antigens in *E. coli*.

## Materials and Methods

### Reagents

Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), and 3-amino-9-ethylcarbazole (AEC) substrate were purchased from Thermo Fisher Scientific. Horseradish peroxidase (HRP)-conjugated goat anti-pig immunoglobulin (IgG) was bought from Sanying Biotech (Wuhan, China). High affinity nickel-nitrilotriacetic acid (Ni-NTA) resin was purchased from GE Healthcare. 3,3',5,5'-tetramethylbenzidine (TMB) was purchased from Sigma-Aldrich. Montanide ISA 50V2 adjuvant was bought from SEPPIC, France. The Bradford Protein Assay Kit and isopropyl- $\beta$ -d-thiogalactoside (IPTG) were bought from Sangon Biotech, Shanghai, China. The plasmid pET28a-gE expressing amino acid residues 51-255 of gE protein was previously constructed in our laboratory. PRV field strain TY-Henan-2014 was isolated from an outbreak-affected swine herd in 2014 (Wang et al. 2015). Clinical serum samples were collected from swine farms in different regions of Henan province, China.

### Protein expression and purification

*E. coli* BL21(DE3) cells bearing pET28a-gE were cultured in Luria-Bertani (LB) liquid media at 37°C for 2-3 h until the optical density of the cell culture at a wavelength of 600 nm ( $OD_{600}$  value) reached 0.4-0.6. Then, the expression of gE protein was induced with IPTG by shaking at 220 rpm at indicated temperature for a fixed time. Next, the cells were pelleted by centrifugation at 6000 g for 20 min at 4°C, and resuspended in Tris buffered saline (TBS, pH 8.0) containing 20 mM Tris and 500 mM NaCl (binding buffer). After ultra-sonication on ice, the supernatant was harvested by centrifugation at 12000 g for 20 min at 4°C and filtered through a 0.45  $\mu$ m nitrocellulose filter. Then, recombinant gE protein with a hexahistidine ( $His_6$ )-tag was purified through Ni-NTA affinity chromatography as follows. Firstly, the affinity column was equilibrated with binding buffer and loaded with the collected supernatant. Secondly, the column was washed with binding buffer containing 40 mM imidazole (wash buffer). Thirdly, gE protein was eluted with binding buffer containing 500 mM imidazole (elution buffer). The collected samples were analyzed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and dialyzed against 20 mM TBS (pH 8.0). A densitometric analysis was performed using the Image J software to analyze protein purity. The sample with the highest purity was used for animal immunization.

### Preparation of swine hyperimmune sera against gE protein

Three 20-day-old piglets were immunized four times with the purified gE protein. The immunization procedure was as follows: the antigen (30  $\mu$ g per pig) was mixed with 50V2 adjuvant and emulsified for 15 min on an ice bath. Each pig then received a 1 mL intramuscular injection of the emulsion. The immunization was performed at 4-week intervals. After the second immunization, blood samples were collected from the anterior vena cava and serum antibody titers were monitored. Following four immunizations, the piglet with the highest antibody titer was selected, euthanized, and exsanguinated. The collected blood was kept at 4°C overnight and then separated by centrifugation at 4000 rpm for 20 min to obtain the hyperimmune serum.

### Enzyme-linked immunosorbent assay (ELISA)

The ELISA for evaluating the immunoreactivity of gE protein was performed as previously described (Wang et al. 2016, Wang et al. 2024). Briefly, microtiter

plates manufactured from polyvinyl chloride were coated with 50  $\mu$ L of gE protein (2.0  $\mu$ g/mL) in carbonate buffered saline (CBS, pH 9.6) and incubated overnight at 4°C. Following three washes with phosphate buffered saline containing 0.05% Tween-20 (PBST), the plates were blocked with 5% skimmed milk at 37°C for 1 h. Next, clinical swine sera diluted 1:200 in *E. coli* cell lysate was added in duplicate and incubated with the plates at 37°C for 30 min. After washing six times with PBST, the plates were incubated with 50  $\mu$ L HRP-conjugated goat anti-pig IgG at 37°C for 30 min. Then, the plates were washed again and finally incubated with TMB for color development. After 10 min of incubation at room temperature, the enzymatic reaction was stopped with the addition of 2 M  $H_2SO_4$  and the optical density of the colored product at a wavelength of 450 nm ( $OD_{450}$ ) was quantified with an automatic microplate reader (Bio-Tek Instruments, Inc., Winooski, VT, USA). All data were measured in triplicate, and the mean  $OD_{450}$  value (X) and standard deviation (SD) were calculated. A positive reaction was confirmed when the ratio between the  $OD_{450}$  value of the sample and the  $OD_{450}$  value of the negative control was over 2.1 (Crowther 2000).

### Immunoperoxidase monolayer assay (IPMA)

The IPMA for evaluating the reactivity of gE-specific hyperimmune sera with PRV-infected cells was performed as previously described (Wang et al. 2023). Briefly, baby hamster kidney cells (BHK-21) were cultured in DMEM media containing 10% FBS and allowed to grow into a confluent monolayer within 24 h. After washing twice with DMEM, the cells were infected with PRV field strain TY-Henan-2014 and grown in DMEM containing 2% FBS for 24 h. Then, the cells were washed with PBS three times and fixed with cold methanol containing 2%  $H_2O_2$  at room temperature for 15 min. After the plates were blocked with 5% skimmed milk at 37°C for 1 h, clinical swine sera or gE-specific hyperimmune swine sera was added and allowed to incubate with the cells at 37°C for 30 min. After washing six times with PBST, HRP-conjugated goat anti-pig IgG were added and incubated with the plate at 37°C for 30 min. Finally, AEC substrate buffer was added for color development for 5 min before the addition of double distilled water to wash the plates and stop the color reaction. The wells were then observed under a light microscopy and the presence of red brown precipitates indicates a positive reaction.

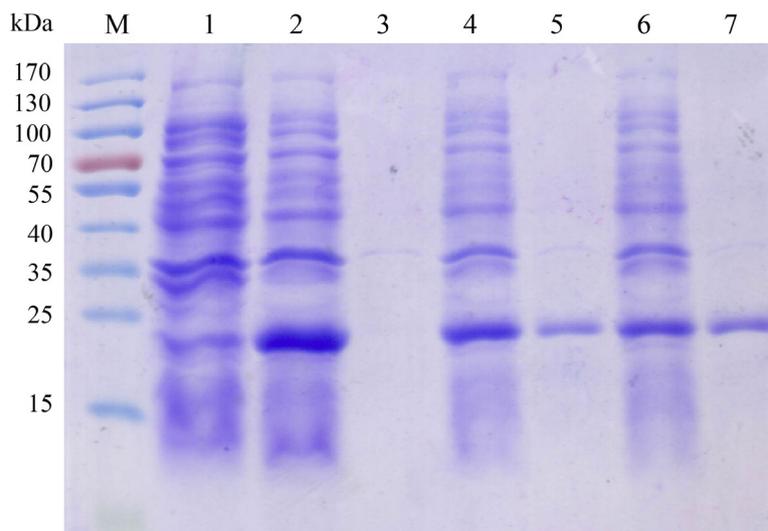


Fig. 1. Optimization of induction temperature for the expression of the gE protein of pseudorabies virus (PRV). Lane M, pre-stained protein molecular weight marker (kDa); Lane 1, uninduced cells; Lane 2, 4, and 6, supernatants from cells induced at 25°C, 30°C, and 37°C respectively; Lane 3, 5, and 7, precipitates from cells induced at 25°C, 30°C, and 37°C respectively.

## Results

### Optimization of the induction temperature for expression of water-soluble gE protein

To circumvent the challenge of inclusion body formation, a common bioprocessing approach is to reduce the host's growth temperature after the induction of recombinant protein expression (Bjerga et al. 2016, Huleani et al. 2021). To optimize the expression of the gE protein, we evaluated its solubility at different induction temperatures. Analysis of the supernatant revealed a marked decrease in soluble gE yield as the induction temperature was raised from 25°C to 30°C and 37°C. Conversely, inclusion body formation increased with temperature (Fig. 1). Since near-complete solubility was achieved at 25°C, this temperature was identified as optimal for maximizing the yield of correctly folded gE protein.

### Determination of the IPTG concentration for expression of water-soluble gE protein

IPTG concentration can modulate recombinant protein expression and solubility by controlling induction strength (Terpe 2006). To determine the optimal condition for gE protein expression at 25°C, we evaluated a range of IPTG concentrations (0.4-1.0 mM). The results revealed that while soluble yield plateaued above 0.4 mM, no insoluble gE was observed in the pellet at any concentration within this range (Fig. 2). Since 0.4 mM was sufficient for maximal soluble production, it was identified as the optimal working concentration of IPTG.

### Determination of the optimal induction time for expression of water-soluble gE protein

The duration of IPTG induction is a critical parameter that directly impacts the total yield, solubility, and bioactivity of a target recombinant protein (Hayat et al. 2018). To determine the optimal time window for gE protein expression, a time-course experiment was conducted at a fixed temperature of 25°C and an IPTG concentration of 0.4 mM. Samples were collected at various time points (2, 4, 6, 8, 10, 16, and 20 h) post-induction. The results demonstrated that the yield of soluble gE protein in the supernatant increased as the induction time was extended from 2 to 8 h (Fig. 3). Notably, no gE protein was detected in the insoluble pellet at any of the time points analyzed, indicating excellent solubility under these conditions. However, a critical plateau was observed, as further increasing the induction time beyond 8 h (up to 20 h) did not result in any additional increase in protein yield. Therefore, based on the maximal accumulation of water-soluble gE protein, the optimal induction time was determined to be 8 h.

### Purification of water-soluble gE protein

The recombinant gE protein was purified using Ni-NTA affinity chromatography (Fig. 4). SDS-PAGE analysis confirmed that water-soluble gE protein was captured by the Ni-NTA resin, demonstrating that the His<sub>6</sub>-tag remained accessible for binding under the established expression conditions. The final yield of the purified gE protein was estimated to be 35 mg per liter of cell culture, as determined by Bradford assay.

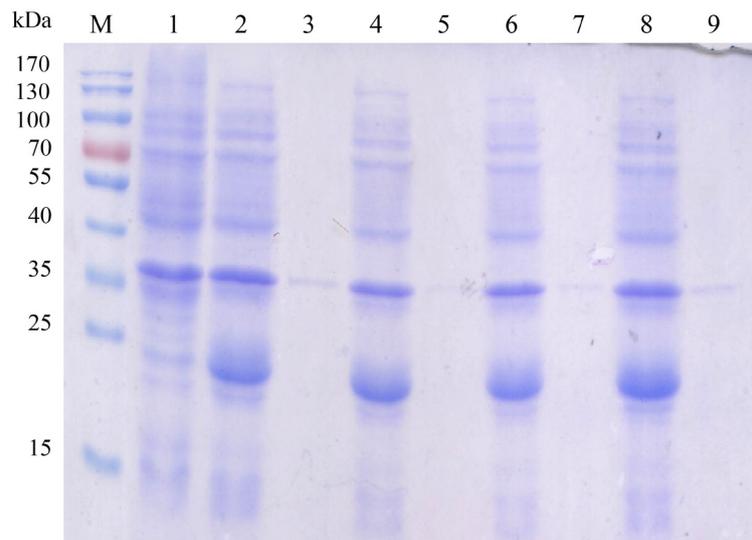


Fig. 2. Determination of the working concentration of isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG). Lane M, pre-stained protein molecular weight marker (kDa); Lane 1, uninduced cells; Lane 2, 4, 6, and 8, supernatants from cells induced with 0.4 mM, 0.6 mM, 0.8 mM, and 1.0 mM IPTG, respectively; Lane 3, 5, 7, and 9, precipitates from cells induced with 0.4 mM, 0.6 mM, 0.8 mM, and 1.0 mM IPTG, respectively.

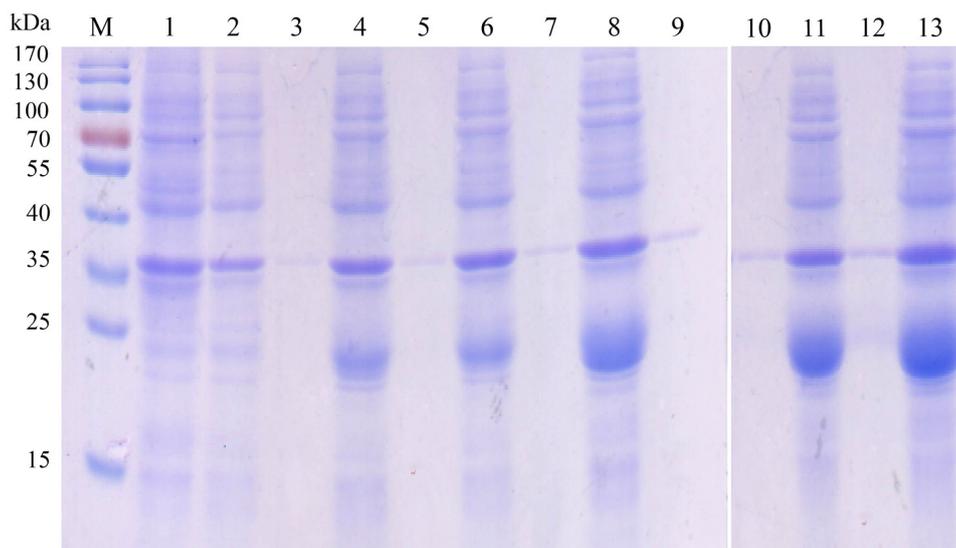


Fig. 3. Determination of the optimal induction time for the expression of gE protein. Lane M, pre-stained protein molecular weight marker (kDa); Lane 1, uninduced cells; Lane 2, 4, 6, 8, 11, and 13, supernatants from cells induced for 2 h, 4 h, 6 h, 8 h, 10 h, and 20 h, respectively; Lane 3, 5, 7, 9, 10, and 12, precipitates from cells induced for 2 h, 4 h, 6 h, 8 h, 10 h, and 20 h, respectively.

The densitometric analysis showed that the purity of gE protein in the tube ranged from 41.6% to 73.4%.

#### Evaluation of the antigenicity of water-soluble gE protein

To assess the immunoreactivity of the water-soluble gE protein, we performed an indirect ELISA using PRV-positive swine sera. The recombinant gE protein was serially diluted (two-fold from 10  $\mu$ g/mL to 0.15  $\mu$ g/mL) as the coating antigen and tested against a fixed dilution (1:200) of the PRV-positive swine sera. The results demonstrated that a coating concentration

as low as 0.62  $\mu$ g/mL could be clearly detected, yielding a positive-to-negative (P/N) ratio of 2.55 (Fig. 5). This confirms that the water-soluble gE protein purified under native conditions retains robust immunoreactivity.

The immunogenicity of the water-soluble gE protein was evaluated by generating specific hyperimmune sera in swine. IPMA demonstrated that swine hyperimmune sera specific for the gE protein reacted with PRV-infected BHK-21 cells, confirming that the water-soluble gE protein retained the native epitopes found on the virion (Fig. 6).

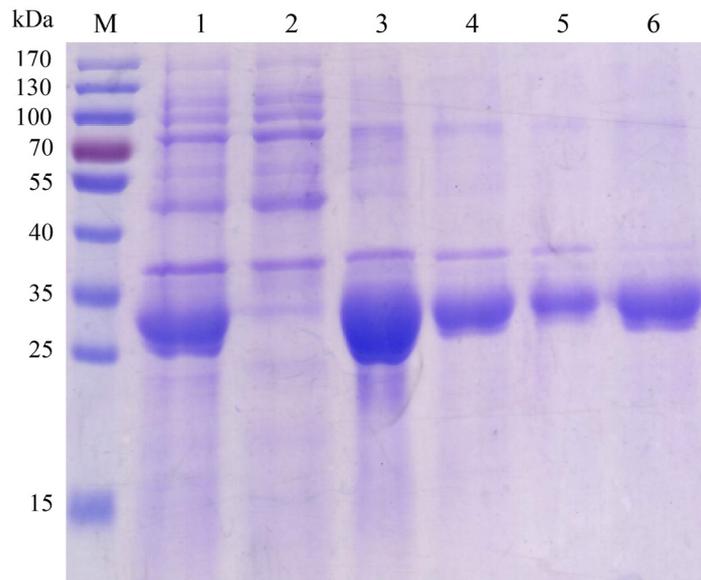


Fig. 4. Purification of gE protein using Ni-NTA affinity chromatography. Lane M, pre-stained protein molecular weight marker (kDa); Lane 1, supernatants from induced cells; Lane 2, flow-through of the sample; Lane 3-6, eluents of gE protein corresponding to a purity of 41.6%, 60.4%, 72.2% and 73.4%, respectively.

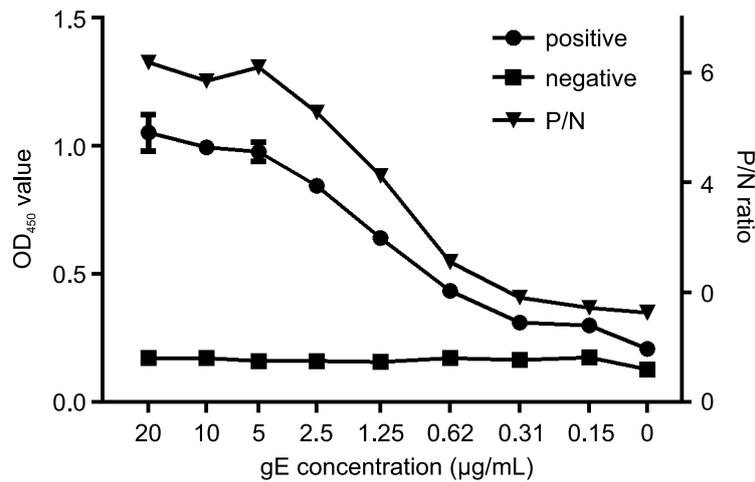


Fig. 5. Reactivity of gE protein with PRV-positive swine sera in ELISA. The immunoreactivity of the water-soluble gE protein was evaluated using indirect ELISA in which serially two-fold diluted gE protein reacted with clinical PRV-positive and PRV-negative swine sera, respectively.

## Discussion

Through systematic optimization of cultivation parameters, we established a simple and efficient method for the high-yield expression of water-soluble gE in *E. coli*. The optimal conditions (25°C, 0.4 mM IPTG, 8 h) effectively suppressed inclusion body formation, yielding approximately 35 mg/L of purified protein. Crucially, this water-soluble gE protein retained native epitopes, as demonstrated by its strong immunoreactivity with PRV-positive swine sera in ELISA and its ability to generate hyperimmune swine sera that specifically recognized wild-type PRV in infected cells. This non-genetic approach bypasses the high costs and extended timelines of eukaryotic cell

culture and avoids the genetic complexity of chaperone systems, offering a streamlined, economically viable path for the large-scale production of diagnostic antigens.

In the optimization process, we found that the solubility of the gE protein was highly dependent on the induction temperature. While nearly all protein was soluble at 25°C, higher temperatures (30°C and 37°C) led to a significant decrease in soluble yield and a concomitant increase in inclusion body formation (Fig. 1). Consequently, 25°C was determined to be the optimal induction temperature for maximizing the expression of water-soluble gE protein. We speculate that by lowering the metabolic rate and the speed of translation, a reduced temperature might provide a longer temporal

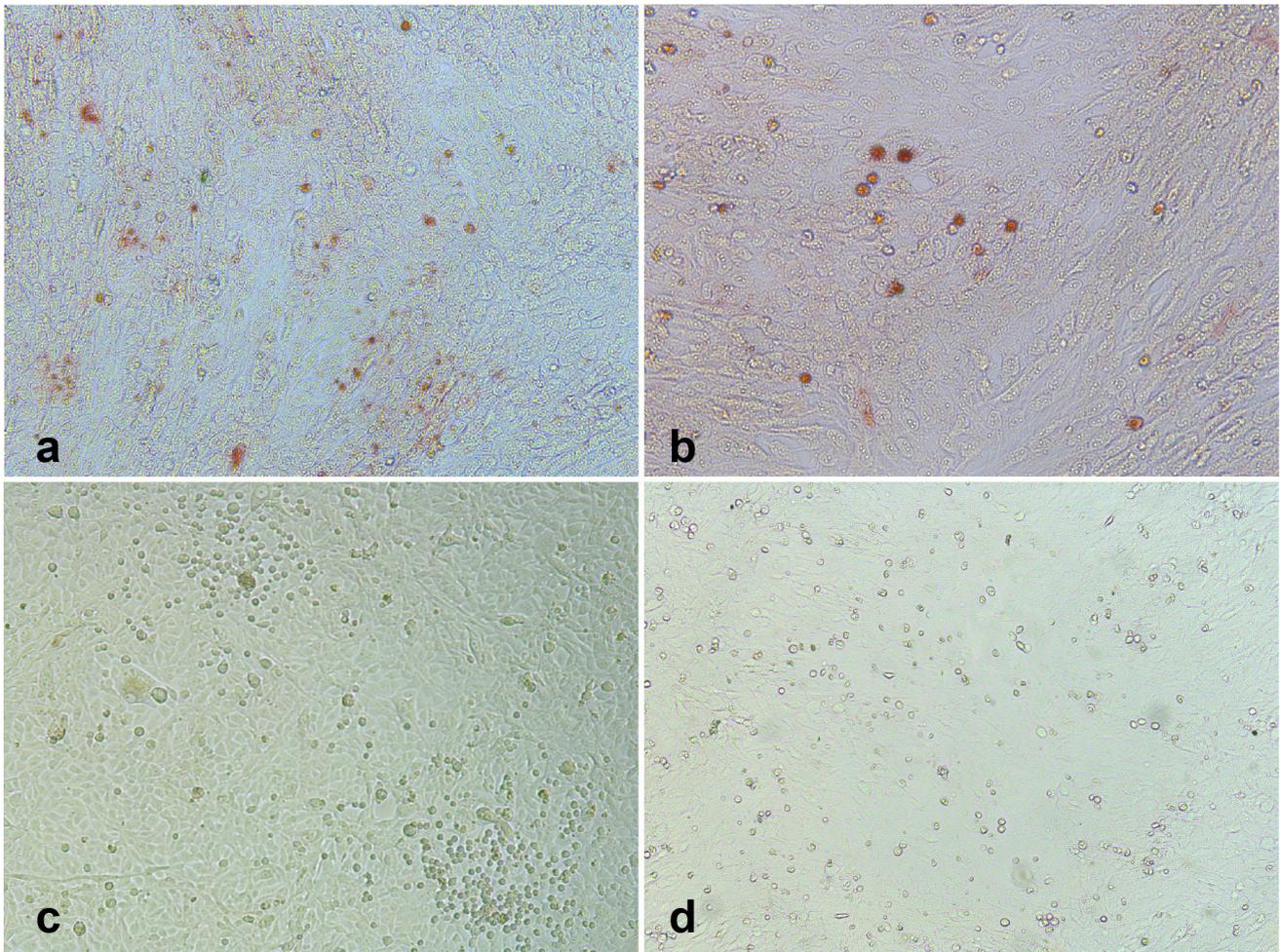


Fig. 6. Reactivity of gE protein-specific swine sera with PRV-infected cells in IPMA. a. the reaction between clinical PRV-positive swine sera and PRV-infected BHK-21 cells; b. the reaction between gE protein-specific hyperimmune swine sera and PRV-infected BHK-21 cells; c. the reaction between PRV-negative swine sera and PRV-infected BHK-21 cells; d. the reaction between pre-immune swine sera and PRV-infected BHK-21 cells.

window for productive folding and suppress non-specific interactions and aggregation that lead to inclusion body formation. Meanwhile, the decrease in thermal energy attenuates the strength of non-specific hydrophobic interactions, which are a primary driver of the intermolecular aggregation that causes inclusion body formation (Kaur et al. 2018, Upadhyay et al. 2012).

Employing a low IPTG concentration has been reported to mitigate the metabolic burden imposed by the T7 expression system (Gupta and Shukla 2015, Lozano Terol et al. 2021). In this study, screening of IPTG concentrations (0.4-1.0 mM) for gE expression at 25°C showed that the soluble yield in the supernatant reached a plateau at 0.4 mM, with no significant improvement at higher concentrations. Critically, no gE was found in the insoluble pellet across the entire range tested. On this basis, 0.4 mM was chosen as the optimal IPTG concentration. It is speculated that by moderating the transcription of target mRNA, low inducer concentration ensured a slower, more manageable flux of nascent polypeptides entering the cellular folding

machinery, which prevented the saturation of chaperones and folding enzymes, thus effectively minimizing misfolding and aggregation (Eskandari et al. 2024, Hayat et al. 2018).

The time-course experiment identified a distinct plateau in soluble gE yield at 8 h, defining the optimal harvest point. This plateau indicates that the *E. coli* host's recombinant expression capacity had been fully utilized under these specific conditions. Extending the process beyond this window not only fails to enhance productivity but also introduces practical drawbacks, including higher energy consumption and an elevated risk of product degradation, thereby reducing the overall efficiency and cost-effectiveness of the production process.

To be noted, the optimization in this study was performed using a one-factor-at-a-time (OFAT) approach. While this method efficiently identified a highly productive condition for soluble gE expression, we recognize that statistical design-of-experiment methods, such as response surface methodology (RSM), could provide

a more detailed model of parameter interactions and are recommended for future work focused on fine-tuning and scaling up this process.

Following purification by Ni-NTA affinity chromatography, the recombinant gE protein was obtained with a rough yield of 35 mg per liter of cell culture. The effective binding of the soluble gE to the resin confirmed that the His<sub>6</sub>-tag was co-exposed and functional, validating the integrity of the recombinant construct under the optimized expression protocol. Based on the SDS-PAGE analysis and densitometry results presented in Fig. 4, the purity of the gE protein following a single-step Ni-NTA affinity purification ranged from 41.6% to 73.4%. While this level of purity was not optimal for structural studies or as a definitive reference standard, the active water-soluble gE antigen produced here may serve as an excellent starting material for downstream polishing to meet required purity specification. Additionally, while SDS-PAGE under denaturing conditions indicated a predominant band at the expected molecular weight, size-exclusion chromatography (SEC) analysis was not performed in this study to precisely determine the oligomeric state (e.g., monomer vs. dimer) of the purified soluble gE protein in its native form.

The immunoreactivity of water-soluble gE protein was examined in ELISA, which showed that a coating concentration as low as 0.62 µg/mL was able to achieve a clear detection with a high P/N ratio, underscoring the diagnostic potential of the purified gE protein. This potent reactivity with polyclonal sera from infected animals confirms that the protein preserves multiple B-cell epitopes which are critical for specific antibody recognition and immunodiagnostic applications.

The IPMA provided the most definitive evidence for the structural and antigenic authenticity of water-soluble gE protein. The key finding was that hyperimmune sera targeting water-soluble gE protein robustly recognized wild-type PRV in its natural biological state-infecting host cells. This conclusively proved that the epitopes presented by the gE antigen were immunologically equivalent to those on the native gE within the virion. This epitope-level concordance is the fundamental prerequisite for creating a highly specific discriminatory diagnostic reagent, as it ensures that antibodies induced by natural infection (against the wild-type gE) will be reliably detected, thereby enabling clear distinction from animals vaccinated with gE-deleted strains. Although solubility in a bacterial lysate does not automatically confer a natively folded, monodisperse, and functional state, the definitive antigenicity validation by ELISA and IPMA confirmed that the water-soluble gE protein possessed the correct conformational epitopes necessary for its diagnostic function. Misfolded

or aggregated proteins would be unlikely to show strong reactivity with infection-induced polyclonal antibodies or to elicit specific antisera capable of recognizing wild-type virus in infected cells.

Previous studies have expressed the gE protein using yeast expression systems and baculovirus/insect cell expression systems, but the costs were relatively high (Ao et al. 2003, Gómez-Sebastián et al. 2008, Wu et al. 2017). When using the *E. coli* expression system to produce the gE protein, inclusion body expression often occurs, requiring an uncontrollable renaturation process to obtain active proteins (Morenkov et al. 1999, Yong et al. 2005). In this study, the systematic condition optimization was applied to the expression of water-soluble gE protein. The strategy of fine-tuning cultivation parameters, a non-genetic approach, offers significant practical advantages over more complex alternatives. By avoiding chaperone co-expression and large fusion tags, we eliminated associated challenges such as genetic instability, unpredictable effects on protein function, and cumbersome tag removal. Beyond the specific case of PRV gE, the systematic optimization strategy presents a generalizable framework for achieving soluble expression of other recalcitrant water-soluble viral antigens or multi-domain extracellular proteins in *E. coli*. While the water-soluble gE protein showed excellent reactivity with PRV-specific antibodies, this study has limitations regarding full diagnostic validation. Future work is necessary to: (i) carry out long-term stability studies and analysis of degradation profiles under various storage conditions, (ii) assess potential cross-reactivity with sera from animals infected with other common swine pathogens, particularly other herpesviruses; and (iii) perform a comparison of the sensitivity and specificity of a gE protein-based ELISA and a commercially available gold-standard test using a large, well-characterized serum panel. The production strategy described herein provides a viable and economical source of antigen for such downstream assay development and validation studies.

## Acknowledgements

This work was supported by the Training Program for Young Backbone Teachers in Higher Education Institutions of Henan Province (2025GGJS089), the Henan Provincial Key Scientific Programs for Universities (25A330004), and the Henan Provincial Student Innovation and Entrepreneurship Training Program (202410472013).

## Author Declarations

### Ethics approval

The animal immunization protocol was approved by the Animal Care and Use Committee of Henan Academy of Agricultural Sciences (Number: HAAS-2014112024). All procedures were conducted in compliance with national and institutional guidelines for the ethical care and use of laboratory animals. The use of three piglets was necessary for polyclonal antibody production. The animals were housed under standard conditions with access to food and water. Immunizations and blood collections were performed using standard techniques and with measures to minimize stress and discomfort by a trained professional.

### Use of generative artificial intelligence

No artificial intelligence tools were used in the preparation of the manuscript.

### Conflict of interest

The authors declared that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## References

- Ai J, Weng S, Cheng Q, Cui P, Li Y, Wu H, Zhu Y, Xu B, Zhang W (2018) Human endophthalmitis caused by pseudorabies virus infection, China, 2017. *Emerg Infect Dis* 24: 1087-1090.
- Ao J, Wang J, Chen X, Wang X, Long Q (2003) Expression of pseudorabies virus gE epitopes in *Pichia pastoris* and its utilization in an indirect PRV gE-ELISA. *J Virol Methods* 114: 145-150.
- Bjerga GE, Lale R, Williamson AK (2016) Engineering low-temperature expression systems for heterologous production of cold-adapted enzymes. *Bioengineered* 7: 33-38.
- Bo Z, Li X (2022) A review of pseudorabies virus variants: genomics, vaccination, transmission, and zoonotic potential. *Viruses* 14: 1003.
- Bude SA, Lu Z, Zhao Z, Zhang Q (2024) Pseudorabies virus glycoproteins E and B application in vaccine and diagnosis kit development. *Vaccines (Basel)* 12: 1078.
- Costa SJ, Almeida A, Castro A, Domingues L, Besir H (2013) The novel Fh8 and H fusion partners for soluble protein expression in *Escherichia coli*: a comparison with the traditional gene fusion technology. *Appl Microbiol Biotechnol* 97: 6779-6791.
- Crowther JR (2000) The ELISA guidebook. In: *Methods Mol Biol*. The Humana Press, USA, pp 1-448.
- Eskandari A, Nezhad NG, Leow TC, Rahman MB, Oslan SN (2024) Essential factors, advanced strategies, challenges, and approaches involved for efficient expression of recombinant proteins in *Escherichia coli*. *Arch Microbiol* 206: 152.
- Gómez-Sebastián S, Pérez-Filgueira DM, Gómez-Casado E, Nuñez MC, Sánchez-Ramos I, Tabarés E, Escribano JM (2008) DIVA diagnostic of Aujeszky's disease using an insect-derived virus glycoprotein E. *J Virol Methods* 153: 29-35.
- Gopal GJ, Kumar A (2013) Strategies for the production of recombinant protein in *Escherichia coli*. *Protein J* 32: 419-425.
- Gupta SK, Shukla P (2015) Advanced technologies for improved expression of recombinant proteins in bacteria: perspectives and applications. *Crit Rev Biotechnol* 36: 1089-1098.
- Hayat SM, Farahani N, Golichenari B, Sahebkar A (2018) Recombinant protein expression in *Escherichia coli* (*E. coli*): what we need to know. *Curr Pharm Des* 24: 718-725.
- Hou Y, Wang Y, Zhang Y, Yu H, Zhao Y, Yi A (2022) Human encephalitis caused by pseudorabies virus in China: a case report and systematic review. *Vector Borne Zoonotic Dis* 22: 391-396.
- Huleani S, Roberts MR, Beales L, Papaioannou EH (2021) *Escherichia coli* as an antibody expression host for the production of diagnostic proteins: significance and expression. *Crit Rev Biotechnol* 42: 756-773.
- Kaur J, Kumar A, Kaur J (2018) Strategies for optimization of heterologous protein expression in *E. coli*: Roadblocks and reinforcements. *Int J Biol Macromol* 106: 803-822.
- Liu Q, Wang X, Xie C, Ding S, Yang H, Guo S, Li J, Qin L, Ban F, Wang D, Wang C, Feng L, Ma H, Wu B, Zhang L, Dong C, Xing L, Zhang J, Chen H, Yan R, Wang X, Li W (2022) A novel human acute encephalitis caused by pseudorabies virus variant strain. *Clin Infect Dis* 74: 756.
- Lozano Terol G, Gallego-Jara J, Sola Martínez RA, Martínez Vivancos A, Cánovas Díaz M, de Diego Puente T (2021) Impact of the expression system on recombinant protein production in *Escherichia coli* BL21. *Front Microbiol* 12: 682001.
- Ma L, Wang Y, Wang X, Zhang M, Zhu M (2024) An isothermal recombinase polymerase assay coupled with lateral flow dipstick for differentiation of pseudorabies virus wild isolates and gE-deleted vaccine strains. *Pol J Vet Sci* 27: 621-630.
- Martínez-Alonso M, Vera A, Villaverde A (2007) Role of the chaperone DnaK in protein solubility and conformational quality in inclusion body-forming *Escherichia coli* cells. *FEMS Microbiol Lett* 273: 187-195.
- Mettenleiter TC (2000a) Aujeszky's disease (pseudorabies) virus: the virus and molecular pathogenesis – state of the art, June 1999. *Vet Res* 31: 99-115.
- Mettenleiter TC (2020b) Aujeszky's disease and the development of the marker/DIVA vaccination concept. *Pathogens* 9: 563.
- Morenkov O, Fodor N, Fodor I (1999) Indirect ELISAs based on recombinant and affinity-purified glycoprotein E of Aujeszky's disease virus to differentiate between vaccinated and infected animals. *Acta Vet Hung* 47: 137-150.
- Ou J, Cai S, Zheng F, Lu G, Zhang G (2020) Human pseudorabies virus infection: a new threat in China. *J Infect* 80: 578-606.
- Policicova K, Goldsmith K, Rainish BL, Wisner TW,

- Johnson DC (2005) The extracellular domain of herpes simplex virus gE is indispensable for efficient cell-to-cell spread: evidence for gE/gI receptors. *J Virol* 79: 11990-12001.
- Pomeranz LE, Reynolds AE, Hengartner CJ (2005) Molecular biology of pseudorabies virus: impact on neurovirology and veterinary medicine. *Microbiol Mol Biol Rev* 69: 462-500.
- Rosano GL, Ceccarelli EA (2014) Recombinant protein expression in *Escherichia coli*: advances and challenges. *Front Microbiol* 5: 172.
- Serena MS, Geisler C, Metz GE, Corva SG, Mortola EC, Larsen A, Jarvis DL, Echeverrıa MG (2013) Expression and purification of Suid Herpesvirus-1 glycoprotein E in the baculovirus system and its use to diagnose Aujeszky's disease in infected pigs. *Protein Expr Purif* 90: 1-8.
- Terpe K (2006) Overview of bacterial expression systems for heterologous protein production: from molecular and biochemical fundamentals to commercial systems. *Appl Microbiol Biotechnol* 72: 211-222.
- Tischer BK, Osterrieder N (2010) Herpesviruses – a zoonotic threat? *Vet Microbiol* 140: 266-270.
- Upadhyay AK, Murmu A, Singh A, Panda AK (2012) Kinetics of inclusion body formation and its correlation with the characteristics of protein aggregates in *Escherichia coli*. *PLoS One* 7: e33951.
- Wang M, Wang L, Zhao Q (2019) Efficacy evaluation of two live virus vaccines against an emerging pseudorabies virus variant. *Pol J Vet Sci* 22: 639-645.
- Wang Y, Guo J, Qiao S, Li Q, Yang J, Jin Q, Zhang G (2016) GP5 protein-based ELISA for the Detection of PRRSV Antibodies. *Pol J Vet Sci* 19: 495-501.
- Wang Y, Qiao S, Li X, Xie W, Guo J, Li Q, Liu X, Hou J, Xu Y, Wang L, Guo C, Zhang G (2015) Molecular epidemiology of outbreak-associated pseudorabies virus (PRV) strains in central China. *Virus Genes* 50: 401-409.
- Wang YB, Li P, Gao YC, Hao PF, Feng JW, Hu NY, Cao J, Hu JH, Ding K, Wang L (2024) Development of a capsid protein-based ELISA for the detection of PCV2 antibodies in swine serum. *Pol J Vet Sci* 27: 529-529.
- Wang YB, Wang SW, Jin QY, Chen LP, Zhang FQ, Shi JJ, Yin Y, Fan ZX, Liu XY, Wang LP, Li P (2023) Expression of water-soluble nucleocapsid protein of SARS-CoV-2 and analysis of its immunogenicity. *Pol J Vet Sci* 26: 571-579.
- Wingfield PT, Palmer I, Liang SM (2014) Folding and purification of insoluble (inclusion body) proteins from *Escherichia coli*. *Curr Protoc Protein Sci* 78: 6.5.1-6.5.30.
- Wu CY, Wu CW, Liao CM, Chien MS, Huang C (2017) Enhancing expression of the pseudorabies virus glycoprotein E in yeast and its application in an indirect sandwich ELISA. *J Appl Microbiol* 123: 594-601.
- Yong T, Huan-chun C, Shao-bo X, Ya-li Q, Qi-gai H, Yu-qi R (2005) Development of a latex agglutination test using the major epitope domain of glycoprotein E of pseudorabies virus expressed in *E. coli* to differentiate between immune responses in pigs naturally infected or vaccinated with pseudorabies virus. *Vet Res Commun* 29: 487-497.