

# Optimized Expression, Purification of Herpes B Virus gD Protein in *Escherichia coli*, and Production of Its Monoclonal Antibodies

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## Abstract

**Background:** Herpes B virus (BV) is a zoonotic disease caused by double-stranded enveloped DNA virus with cercopithecidae as its natural host. The mortality rate of infected people could be up to 70% with fatal encephalitis and encephalomyelitis. Up to now, there are no effective treatments for BV infection. Among the various proteins encoded by monkey B virus, gD, a conserved structural protein, harbors important application value for serological diagnosis of frequent variations of the monkey B virus.

**Objectives:** This study aimed to express the gD protein of BV in *Escherichia coli* by a recombinant vector, and prepare specific monoclonal antibodies against gD of BV to pave the way for effective and quick diagnosis reagent research.

**Materials and Methods:** The gD gene of BV was optimized by OptimWiz to improve codon usage bias and synthesis, and the recombinant plasmid, pET32a/gD, was constructed and expressed in *E. coli* Rosetta (DE3). The expressed fusion protein, His-gD, was purified and the BALB/c mice were immunized by this protein. Spleen cells from the immunized mice and SP2/0 myeloma cells were fused together, and the monoclonal cell strains were obtained by indirect enzyme-linked immunosorbent assay (ELISA) screening, followed by preparation of monoclonal antibody ascetic fluid.

**Results:** The optimized gD protein was highly expressed in *E. coli* and successfully purified. Five monoclonal antibodies (mAbs) against BV were obtained and named as 4E3, 3F8, 3E7, 1H3 and 4B6, and with ascetic fluid titers of  $2 \times 10^6$ ,  $2 \times 10^5$ ,  $2 \times 10^5$ ,  $2 \times 10^3$  and  $2 \times 10^2$ , respectively. The 1H3 and 4E3 belonged to the IgG2b subclass, while 3E7, 3F8 and 4B6 belonged to the IgG1 subclass.

**Conclusions:** The cell lines obtained in this work secreted potent, stable and specific anti-BV mAbs, which were suitable for the development of herpes B virus diagnosis reagents.

**Keywords:** Herpes B Virus (BV), gD Protein, Optimized Expression, Protein Purification, Monoclonal Antibody

## 1. Background

*Herpesviridae* is a family of viruses with double-stranded DNA. The B virus (*Cercopithecine herpesvirus 1*, or herpes B virus) and *Cercopithecine herpesvirus 2* (CeHV-2) are primate herpes viruses that belong to the alpha-herpesvirus subfamily, and are closely related to herpes simplex virus 1 (HSV-1). The coated particle harbors a linear double-stranded DNA genome of about 157 kb (1-4). Herpes B virus (BV) is a zoonotic virus and its natural host is mainly cercopithecidae, although BV infections are usually mild or unapparent in macaques, yet lead to fatal encephalitis and encephalomyelitis in humans (2, 5, 6). Previous studies (7) have shown that BV is the only known agent that could infect humans among the 35 herpesviruses confirmed in nonhuman primates. In the early stages of infection, if not treated by antiviral therapy, the infection would have a high mortality.

As nonhuman primates are the main experimental an-

imals for biomedical research, development of an effective method for surveillance of BV infection is essential for the establishment of BV-free monkey colonies and reduction of the risk for laboratory workers, animal handlers and researchers (8). Unfortunately, in most cases, usage of cell culture or polymerase chain reaction (PCR) for an immediate diagnosis of BV infection was impossible, for the existence of similarity between BV and other alpha-herpesviruses. Herpes B virus spends a life-long lurking in sensory ganglia of macaques and rarely reactivates (9). The detection of serum antibodies to BV proteins was used for diagnosis of BV infections in humans and monkeys. Thus, serological test of anti-BV antibodies is the only effective way for the surveillance of infected animals.

Currently, a few serological test techniques are being used to detect the viral infection, relying on recombinant proteins and cell lysates of BV (10). To identify infected animals, the solubilized HSV-infected cell antigen was de-

veloped and used for ELISA and other rapid serological tests (11-13), with subsequent western-blotting confirmation to identify specific targets that could be immunoreactive with serum antibodies (14). Because of high cross-reactivity with BV proteins, HSV type 1 (HSV-1) and HSV-2 are not specific enough to clearly identify BV infections in herpes simplex virus (HSV) positive humans by this method (15, 16). Among the twelve glycoproteins, which contained in the viral envelope, four had proven with high immunogenicity, gB, gD, gC and mgG (3, 10, 17), the primary targets of IgG antibody responses of the in patients, who were infected by the HSV-1 or HSV-2 have been confirmed as the nucleocapsid complex p40, and the major capsid protein VP5 (6, 16, 18-20), it would be a complex task to differentiate them.

Most importantly, BV is a biosafety level 4 (BSL-4) pathogen; currently BV-infected cell lysates are used as a diagnostic antigen in serological tests and only maximum containment laboratories (BSL-4) have the qualification to produce it, this requirement limits the amount of facilities, which provide the antigen. When compromising outcome measures based on assays using these antigens, the antigens may also suffer from lot-to-lot variation. The glycoprotein D (gD) of BV is a 35-kDa glycoprotein identified in the BV envelope and is among the main surface antigens shown to elicit antibodies in sera of infected animals.

It has a high homology with HSV-1; its amino acid sequence is 56% - 58% identical to that of HSV-1 gD (1). Moreover, BV gD was similar with glycoprotein D of HSV-1 and HSV-2, and is one of the immunodominant envelope proteins that can elicit humoral and cellular immunity in animals and human individuals; the gD protein has been considered as a good immunogen (6, 21). Finally, BV gD protein could bind to human nectin-1 receptors for cell-cell fusion and virus entry (22, 23). Therefore, in B virus infection the gD has been identified as a multifunctional protein, and production of mAbs against gD could also provide an effective tool for elucidation of the exact function of BV glycoprotein gD.

## 2. Objectives

The aim of the present study was to prepare anti-BV mAbs, which is for the diagnosis of BV infection, and provide a foundation for the development of rapid and specific methods.

## 3. Materials and Methods

### 3.1. Cell Lines

The mouse myeloma cell line, SP2/0, was cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (Gibco, USA) at 37°C in a humidified atmosphere in the presence of 5% CO<sub>2</sub> (24). *Escherichia coli* Rosetta (DE3) competent cells were prepared using the calcium chloride method and stored at -80°C (25).

### 3.2. Optimization of gD Gene and Construction of Its Expression Vector

The nucleotide sequence for envelope glycoprotein D gene of BV strain E2490 has been deposited in the GenBank database and the gD gene was optimized with the following accession number, NC-004812. The transmembrane region of gG was analyzed by the SOSUI system, as described previously (26). The extracellular domain of viral glycoprotein usually is the key point for eliciting host antibodies. Therefore, the extracellular region of gD was chosen for codon optimization and synthesized. The *EcoRI* and *HindIII* restriction endonuclease (TaKaRa, Japan) sites in the sequence were then introduced to allow ligation of the entire gD sequence into pET-32(+)(Novagen, Germany); an amino-terminal histidine tag contained the prokaryotic expression vector.

### 3.3. The Expression and Purification of gD Recombinant Proteins

DNA sequencing and restriction endonuclease digestion were used to verify the synthesized gD gene before expression. The recombinant plasmids were transformed into *E. coli* Rosetta (DE3) cells, and *E. coli* cells were grown in shaker flasks with LB broth medium containing 100 µg/mL ampicillin at 37°C until optical density (OD) of 0.6 was reached, and then recombinant bacteria were induced using 0.1 M of isopropyl-β-D-thiogalactoside (IPTG) at 37°C for four hours. The induced recombinant bacteria were analyzed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (27). Four hours after induction, the cells were harvested and centrifuged at 9,000 rpm for ten minutes and washed once with phosphate buffered saline (PBS). Subsequently, the cells were subjected to rapid freeze-thaw treatment at -80°C twice and resuspended in PBS buffer. In addition, cell membranes were disrupted by supersonic waves, and precipitated. The supernatants were collected at 9,000 rpm for 10 minutes at 4°C.

The extracted inclusion body proteins and supernatants were treated with 10 × SDS loading buffer and then verification by 12% SDS-PAGE. Inclusions were processed

with urea of 8 M at 4°C cracking overnight. The recombinant His-tagged gD was purified with a column of nickel-nitrilotriacetic acid (Ni<sup>2+</sup>-NTA) agarose resin. Cell pellets harvested from LB broth were resuspended in 20 mL of PBS. The cell pellets were then sonicated for 15 minutes and centrifuged at 12,000 g for 15 minutes. The inclusion bodies were solubilized in buffer A (8 M urea in ddH<sub>2</sub>O) by incubating the solution at 4°C for eight hours.

The solubilized solution was centrifuged for 20 minutes at 12,000g at 4°C. The supernatant was applied to Ni<sup>2+</sup>-NTA column. The unbound proteins were removed by washing buffer (20 mM Tris-HCl, 500 mM NaCl, 5 mM imidazole, pH 7.9, 8 M urea; one times 6 mL). The elution of gD was accomplished with elution buffer (20 mM Tris-HCl, 500 mM NaCl, 200 mM imidazole, pH 7.9, 8 M urea; five times for 5 mL each). Finally, protein elution solutions were dialyzed against PBS (pH 7.4) for six hours. Protein concentration was measured by standard BCA assay (Cowan Biotech, Beijing, China) according to the manufacturer's instructions, and proteins were aliquoted and stored at -80°C (28).

#### 3.4. Western Blot Analysis

To verify the recombinant gD protein expressed in *E. coli* Rosetta (DE3) cells, western blot analysis was performed using the following procedures (29). Three samples of the purified gD protein were subjected to 12% SDS-PAGE and then transferred to polyvinylidene fluoride (PVDF) using a wet transfer apparatus (Bio-Rad). After blocking with PBS containing 0.05% tween-20 (PBST) and 5% skimmed milk for two hours, one of the PVDF membranes was added to the mouse anti-His-tag mAb (Cwbio, Beijing, China) at a dilution ratio of 1:5000 in 5% skimmed milk solution at 37°C for two hours. After washing with PBST, goat anti-mouse IgG conjugated horseradish peroxidase (Santa Cruz Biotechnology, CA) as secondary antibody, was added at a dilution ratio of 1:3000 and incubated at 37°C for one hour, and then incubated with DAB solution (Cwbio, Beijing, China) for 5 - 10 minutes. The second PVDF membrane was added to monkey B virus serum at a dilution ratio of 1: 500 in 5% skimmed milk solution and the third one was added to monkey negative serum at the same dilution ratio, both of them used the goat anti-rhesus IgG (H + L) HRP (Southern Biotech, USA) as a secondary antibody, this procedure was followed by eECL western blot analysis (Cwbio, Beijing, China). The cell culture of pET-32a-gD-Rosetta (DE3) without IPTG induction was used as the negative control.

#### 3.5. Immunization and Generation of Hybridomas

Three six to eight week-old adult BALB/c mice with body weights of about 17 g were purchased from the experimen-

tal animal center of Kunming Medical University. For the initial immunization, three BALB/c mice were immunized with 1:1 mixtures of gD recombinant protein (25 µL of protein per mouse with the addition of PBS to reach a final volume of 150 µL) and complete Freund's adjuvant (Sigma, St. Louis, MO). Pre-immune serum was collected prior to immunization as the negative control. The first two injections were on both sides of the subcutaneous tissue of the back and neck. The last boost was into the intraperitoneal region. Two weeks after each immunization, a blood sample was obtained from the tails of the immunized mice and tested for titers against gD by ELISA. Recombinant protein was used to coat ninety-six-well plates with a concentration of 0.35 µg/well. Each serum sample was diluted from 1:500 to 1:128,000 by 2-fold serial dilutions.

The secondary antibody was HPR-labeled goat-anti-mouse IgG. A positive OD (OD for positive well) > 0.1 and OD positive / OD negative (OD for negative well) ≥ 2.1 were the criteria for positive results. The mouse with the highest serum titer was selected for fusion, whose spleen was removed and splenocytes were fused with the mouse myeloma cell line (SP2/0). According to previous research, 3:1 - 10:1 were the ratio of the spleen cells mixed with SP2/0 cells. After centrifugation, 0.8 mL of 50% PEG1450 was added drop by drop over one minute into mixed cells and after 90 seconds in order to stop the reaction, serum-free RPMI-1640 medium was added. After centrifugation, the fusion cells were resuspended in RPMI-1640 selective medium containing 20% FBS and 1% HAT, and 100 µL/well of this solution was added to 96 well plates already loaded with the feeder cells. The incubation conditions were 37°C and 5% CO<sub>2</sub>. After fusion, when hybridoma cells covered 1/3 of the area of culture wells, indirect ELISA using gD as coating antigen was screened for individual hybridoma clones in the culture supernatant, using the SP2/0 cells culture supernatant as the negative control (13, 30).

Cells in antibody positive wells were sub-cloned in accordance with the limiting dilution method (30). Positive hybridoma cells were transferred from 96 well plates to 24 well plates, and then transferred to six well plates after overgrowing, and were finally transferred to cell culture flasks for incubation. Incubated cells were cryopreserved. The cell culture supernatants were collected for titer detection by ELISA and were preserved at -80°C. Prior to commencement of the study, all experimental protocols that involved animals received approval from the scientific and ethical committee of the Kunming University of Science and Technology (PR. China).

#### 3.6. Preparation and Purification of Ascites

Scalable culture of anti-gD antibody-positive hybridoma cells was conducted. Firstly, six to eight week-old

female BALB/c mice were injected intraperitoneally with 0.5 mL of sterile liquid paraffin. One week later, each BALB/c mouse was injected intraperitoneally with  $5 \times 10^4$  hybridoma cells in 0.5 mL PBS. Ascites of mice with extremely enlarged abdominal circumference were collected for titer detection by ELISA and were preserved at  $-80^\circ\text{C}$ . Immunoglobulin was purified primarily from ascitic fluid using the ammonium sulfate precipitation technique. The concentration of monoclonal antibodies was determined by the BCA assay (Cowin Biotech, Beijing, China) (30).

### 3.7. Antibody Isotype Determination

Immunoglobulin isotypes were determined using the mouse monoclonal antibody isotyping kit (Southern Biotech, SBA clonotyping System-HRP), according to the manufacturer's instructions.

## 4. Results

### 4.1. Plasmid Construction

Referring to the published sequence of monkey BV E2490 strains, the specific 951-bp fragment of *gD* corresponding to the extracellular region (amino acid sequence of 26 - 341) was synthesized by GENEWIZ Inc. (Suzhou, China) DNA synthesizer. The gene sequence was optimized based on the *E. coli* expression system codon preferences, while the amino acid sequence was maintained (Figure 1). The ideal percentage range of GC content of the gene was between 30% and 70%, after optimization; the average GC content was changed to 60% from the original 72%.

*EcoRI* and *HindIII* sites were designed in the primers to facilitate subsequent cloning. The vector of pET32a-*gD* was created by the *gD* gene, which was cloned in the prokaryotic expression vector pET-32a (+) (31). Inducible T7 RNA polymerase promoter was used to drive the expression of *gD* in this plasmid. The recombinant plasmid was verified by colony PCR (Figure 2), restriction enzymes digestion (Figure 3, lane 2) and analyzed by 1% agarose gel electrophoresis and sequenced (32). It was demonstrated that there was no mutation in the amino acid sequence by the sequencing results (data not shown).

### 4.2. Expression and Purification of the *gD* Protein

After cloning the *gD* gene into the prokaryotic expression vector pET-32a (+), the recombinant *gD* protein was successfully expressed at a high level in *E. coli* (33); its molecular weight was about 53 kDa. The protein was purified with  $\text{Ni}^{2+}$ -NTA agarose resin, and was then eluted using various concentrations of imidazole for harvesting *gD*

(Figure 5). The concentration of the purified His-tagged *gD* protein was 0.1 mg/mL, as shown by the results.

The purified protein was confirmed by western blot analysis with anti-His tag mAb and clinical BV positive monkey serum. The results showed that the recombinant *gD* protein revealed a specific band with expected molecular mass of 53 kDa, which was specifically recognized by anti-His monoclonal antibody (Figure 6A), and the picture of the electrophoresis also confirmed that the recombinant protein could specifically react with monkey B virus serum (Figure 6B). However, the negative serum of monkey had weak cross-reaction with the recombinant protein (Figure 6C). In the negative control, empty vector pET-32a (+) could not be induced to express the *gD* protein; it did not have a specific band in the western blot analysis (34).

### 4.3. Preparation of *gD* Protein-Specific mAb

After the fourth immunization, ELISA was used to test the reactivity of sera, from immunized mice by the recombinant *gD* protein (Figure 7). In accordance with the above criteria, the mice could be used for mAbs production (35).

The culture supernatants of hybridoma cells from mAb were screened by indirect ELISA. Five stable positive hybridomas were cloned by three rounds of subclone and screening, designated as 4E3, 3F8, 3E7, 4B6 and 1H3, chosen to produce ascites (35). The immunoglobulin classes and subclasses of 4E3 and 1H3 were isotyped as IgG2b, while the other three mAbs (3F8, 4B6 and 3E7) were IgG1, and the light chains of all the five mAbs belonged to the kappa chain (Table 1) (36). The mAbs in ascites were purified and confirmed by SDS-PAGE (Figure 8). In accordance with the above criteria, the titers of those mAbs ranged from  $10^2$  -  $10^6$  (Table 1). The concentration of monoclonal antibodies is shown in Table 1. The mAb of 4E3 had the most sensitivity in the detection of B virus.

**Table 1.** Characterization of the mAbs Against *gD* Protein

MABs	Isotype	Ascites Titre	Ascites Concentration, mg/mL
4E3	IgG2b, $\kappa$ chain	$10^6$	3.5
3F8	IgG1, $\kappa$ chain	$10^5$	3
3E7	IgG1, $\kappa$ chain	$10^5$	2
1H3	IgG2b, $\kappa$ chain	$10^3$	1.5
4B6	IgG1, $\kappa$ chain	$10^2$	0.9

## 5. Discussion

In biomedical testing, the nonhuman primate research models are particularly valuable. For a wide vari-

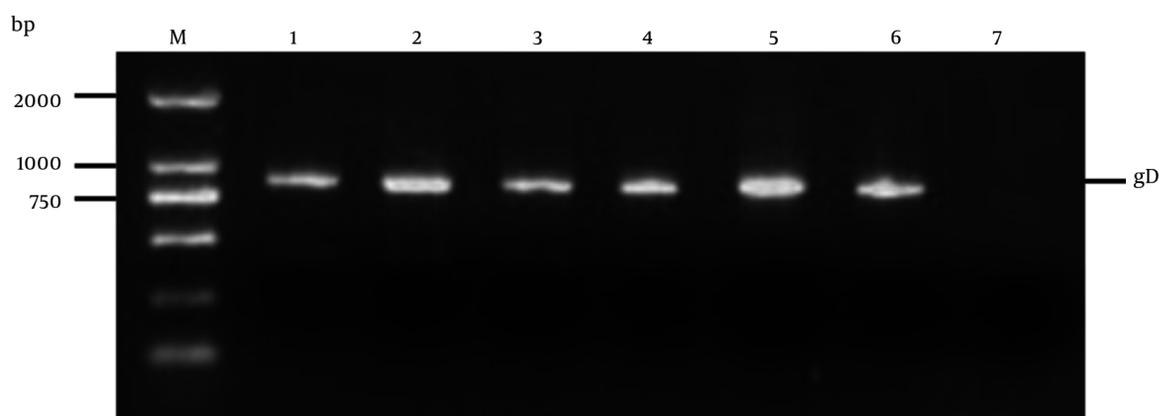
Optimized	1	TATGTGCCGGTGAACGCAGCCTGACACGTGTGAACCCGGGT CGTTTTCGCGGTGCACAT
Original	1	TACGTGCCGGTGGAGCGGTCCTGACTCGGGTGAACCCGGT CGCTTCCGCGGGGCCAC
Optimized	61	CCTCCGCCGCTGAAACAGAAAACCGATCCGCCGGACGTGCGT CGCGTGTATCATGTGCAG
Original	61	CCGCCGCCGCTGGAGCAGAAGACGGACCCCCCGACGTGAGGCGCGTCTACCACGTGCAG
Optimized	121	CCGTTTGTGGAGAACCCTTTTCAAACC CCGAGCGTTCGGTTGCCGT TTAT TACGCAGTG
Original	121	CCGTTTCGTGGAGAACCCGTTCAGACCCCCAGCGTGC CCGTCCCGTGTACTACGCCGTG
Optimized	181	CTGGAACGTGCATGTGCGCAGCGTCTGCTGTGGCCCCGACCGAAGCCGTT CAGGTGGTT
Original	181	CTGGAGCGCGCCTGCCGCAGCGTCTCCTCTGGGCGCGACGGAGGCGGTCCAGGTCTG
Optimized	241	CGGGGAGCCCCAGAGGCGACGCGGTTCGGACGCGCGCTACAACCTCACCGTGGCCTGGTAC
Original	241	CGCACGAGCGACGACTGCGCCA TCCCGATCCTCGTCA TGGAGTACGCGAGTGCCAGTAC
Optimized	301	GATAAACCCTGGGTGCCCTGCCCTGTT CGCAACCTGCCCTCGCTGGAGCTTT TACGATAGC
Original	301	GACAAGCCCTGGGCGCCTGCCCGTCCGCAACCTGCCCGCGCTGGAGCTTCTACGACAGC
Optimized	361	TTCAGCGCAACCGGCGATGATGATCTGGGCT GCTGATGCATGCACCGGCC TTCGAAACC
Original	361	TTCAGCGCCACGGGCGACGACGACCTGGGCT CCTCATGCACGCGCCCGCTTCGAGACC
Optimized	421	GCAGGTACCTATGTTGCGCTGGT TAAGGTGAACGGCTGGGTGGAGGTGACC CAGTTCATC
Original	421	GCGGGGACCTACGTACGCTGGTGAAGGTCAA CCGGTGGGTGGAGGT CACGCAGTTCATC
Optimized	481	TTCGAACATCGCGGTAAAGGCCCGTGCCGCTATACCC TGCCGCTGCGCATTCTGCCGGCA
Original	481	TTCGAGCACCGCGGCAAGGGCCCTGT CGATACACGCTTCCGCTGCGCATCTCCC CGCG
Optimized	541	GCATGTCTGCGCGCACCGGTTTGAA CAGGGTGTGACCGTGATGCCATTGGTATGCTG
Original	541	GCCTGCCTCCGCGCCCCGCTCTCGAGCAGGG CGTAA CCGTGGACGCCATCGGCATGCTC
Optimized	601	CCGCGCTTTATCCCGAAAATCAGCGTATCGTGGCCGTGTACAGTCTGCAGGCCGCCGGT
Original	601	CCGCGGTT CATCCCGGAGAACCAGCGCATCGTGGCCGTCTACAGCCTGCAGGCCGCCGGG
Optimized	661	TGGCATGGTCCTAAGGCCCGTTTACAAGTACCCTGCTGCCG CCGGAAGTTGTGAAACC
Original	661	TGGCACGGCCCAGAGCGCCGTTCACGAGCACCTGCTCCCGCCCAGGTGGTGGAGACG
Optimized	721	GCAAATGTGACCCGTCTGAACTGCA CCGAAGATGAAGATGAGCAGGCCCTGGTGAC
Original	721	GCCAACGT CACCCGGCCC GAGCTGGCCCGGAGGACGAGGACGAGCAGGCC CCGGGCGAC
Optimized	781	GAGCCTGCACCTGCAGTGGCCGACAGCTGCCCGCAATTGGCATGTGCCGAGGCAAGC
Original	781	GAGCCC GCCCCCCGCGTCCGGCCCCGACCTAGCGGTCATACCGGCTAA
Optimized	841	GACGTCACCATCCAGGGCCCCGCCCCGCGCCCTCCG GCCACACGGGCTAA

**Figure 1.** The Comparison of the Optimized and Original Sequence of the Herpes B Virus Gd Gene

ety of biomedical research endeavors the macaques are extremely valuable nonhuman primate models (e.g., a non-human primate model for AIDS) (3). The B virus, which is endemic to rhesus macaques, belongs to the family of herpesviridae. The fatality rate was 80% for infected individuals who were untreated (37). The macaque conventional

populations were naturally infected by the B virus, which is a  $\alpha$ -herpesvirus. A previous study showed the macaques, which carry numerous viruses will harm the researchers and impact the study results.

According to previous studies (38), for the diagnosis of many viral infections, recombinant DNA techniques gen-

**Figure 2.** Identification of the Positive Clone by the Bacteria Polymerase Chain Reaction

M, DL2000 DNA marker; 1-6, randomly selected colony; 7, negative control.

erally play an important role. However, the use of recombinant antigens for BV sero-diagnosis has not been widely investigated, and it has been proposed that the specificity of BV gD is probably a valuable diagnostic reagent for distinguishing herpes B virus infections (23, 39, 40). The A + T and G + C content, rare codons, AT-rich stretches and mRNA secondary structure are factors that could distinctly affect the expression of foreign genes. According to the analysis of the coding sequence of *gD*, we found that it consists of GC-rich material. Therefore, we optimized the gene sequence to be beneficial to the expression of gD. The results demonstrated that the anti-His mAb and BV positive monkey serum could recognize the purified gD protein specifically (Figure 6). MAbs is one of the valuable tools for specific epitopes and characterization of antigenic variation.

The splenocytes from the immunized mice were fused with SP2/0 myeloma cells. After three cycles of subcloning, five mAbs against the gD of B virus were obtained (41). After a test, we determined that our mAbs are stable by comparison with other studies of mAbs (42, 43). We will continue to track their stability. The SDS-PAGE analysis showed that five strains of mAb could have two distinct bands after purification. We used the monkey B virus serum instead of an infected person's viral serum, which is because we did not have access to relevant serum of channels (8). However, we believe it is still possible to obtain reliable results. Sensitive reactions to recombinant antigen gD were shown by the monkey B virus serum and negative serum.

Although an inconsiderable cross-reaction was observed, the WB with the recombinant antigens was established to show specificity for the detection of B virus infections. Unfortunately, we only had HSV-2 virus and without

HSV-1 virus and the standard strain of BVE2490, and the five monoclonal antibodies we screened had specificity and no cross-reactivity with HSV-2 by immunofluorescence assay (Data not shown). Whether they recognize HSV-1 needs further identification. Our research prepared abundant recombinant gD protein antigen, and this could be regarded as significant reference for the BV detection kit. The prepared mAbs could provide basis and a powerful tool for further research of the characteristics and function of envelope protein and its antibody.

#### Acknowledgments

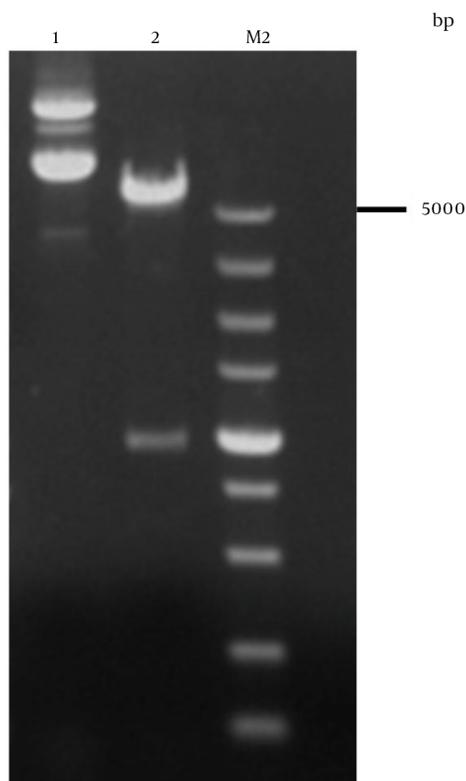
The authors would like to thank Kunming Biomed International for their help with the serum samples.

#### Footnotes

**Authors' Contribution:** Zian Jin and Tao Sun carried out most of the experiments and wrote the manuscript. Jinyang Zhang developed the original idea and critically revised the manuscript. Xueshan Xia, Qiujiang Wei, Yuzhu Song, Qinqin Han, Qiang Chen and Juan Hu helped with the experiments and material preparation. All the authors approved the final manuscript.

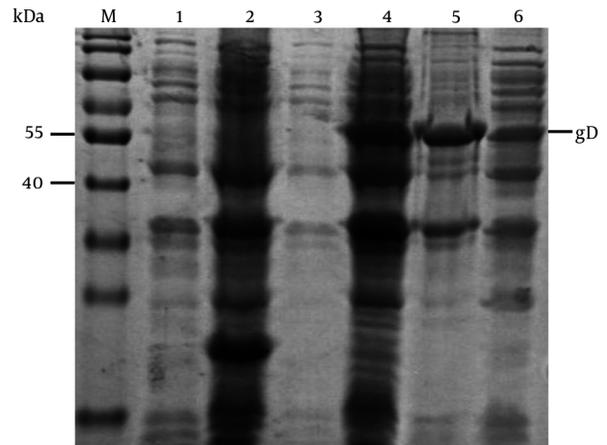
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**Figure 3.** Restriction Enzymes Identification of the Recombinant Plasmid pET-32a-gD



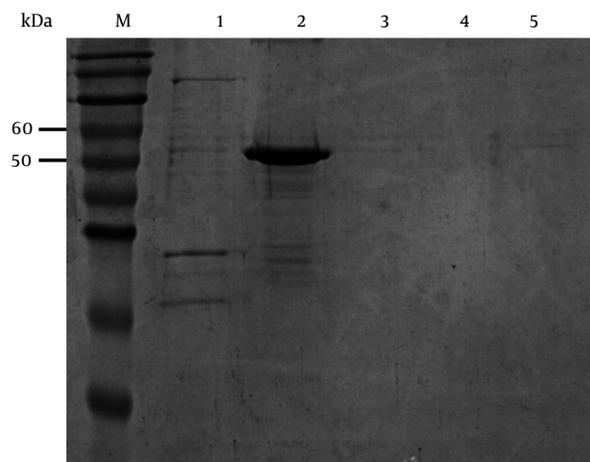
M, DL5000 DNA marker; 1, recombinant plasmid pET-32a-gD; 2, digested product of pET-32a-gD.

**Figure 4.** Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis Analysis of Recombinant Protein Expressed in *Escherichia coli* Rosetta (DE3)



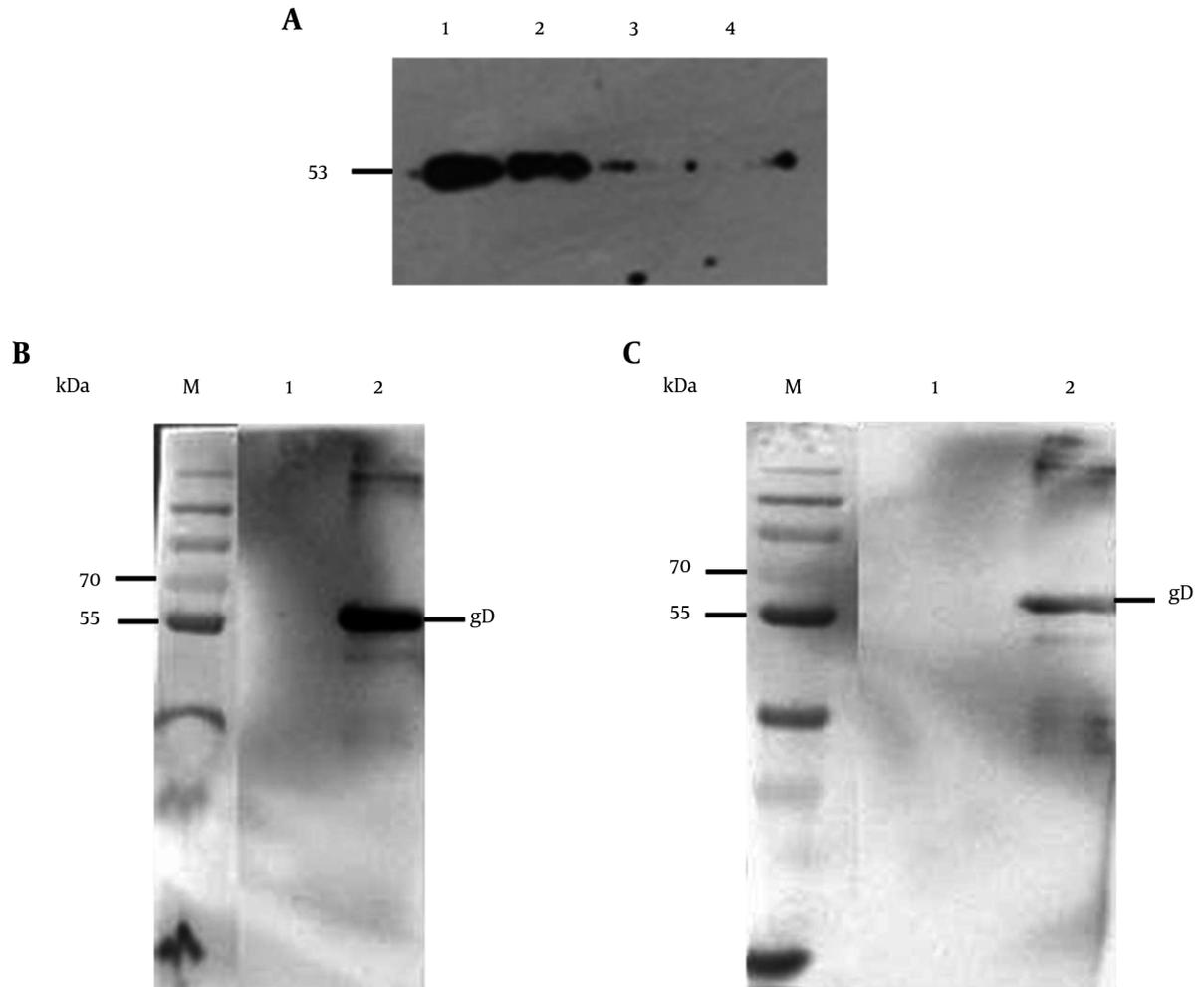
M, protein marker; 1, *E. coli* Rosetta (DE3) containing pET-32a (+) before induction; 2, *E. coli* Rosetta (DE3) containing pET-32a (+) was induced with IPTG; 3, *E. coli* Rosetta (DE3) containing pET-32a-gD before induction; 4, *E. coli* Rosetta (DE3) containing pET-32a-gD induced with IPTG; 5, inclusion bodies of the *E. coli* Rosetta (DE3) with pET-32a-gD induced by IPTG; 6, supernatants of the *E. coli* Rosetta (DE3) lysates with pET-32a-gD induced by IPTG.

**Figure 5.** The Optimization of Recombinant Protein Purification



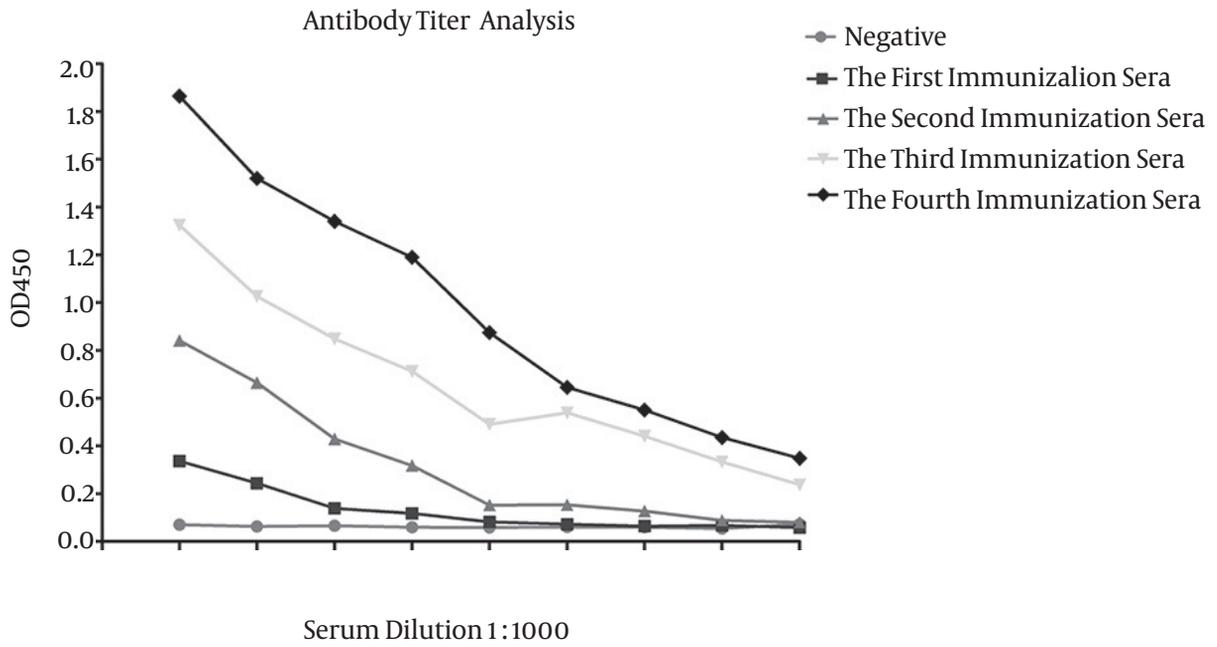
M, protein marker; 1, elution buffer: binding buffer = 2:8; 2, elution buffer: binding buffer = 4:6; 3, elution buffer: binding buffer = 6:4; 4, elution buffer: buffer = 8:2; 5, 100% elution buffer.

**Figure 6.** Identification of Recombinant Protein gD With an-His Tag Antibody and Monkey Herpes B Virus Positive Serum by Western Blot



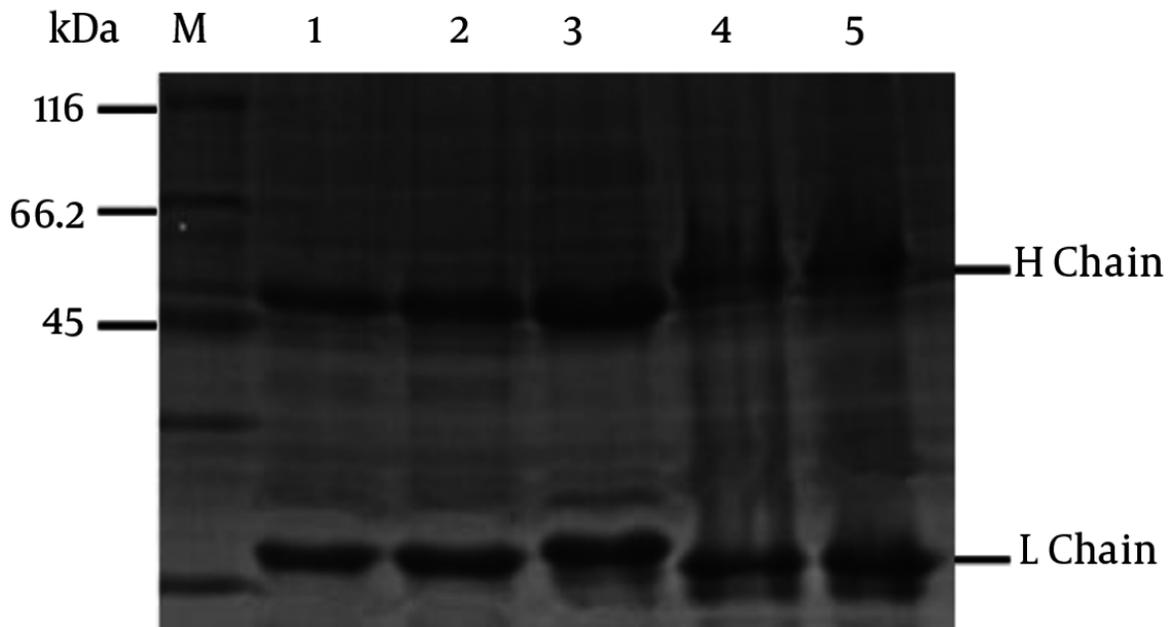
A: 1, purified gD protein from *E. coli* Rosetta (DE3) containing pET-32a-gD; 2, IPTG induced *E. coli* Rosetta (DE3) containing pET-32a-gD; 3, non-induced *E. coli* Rosetta (DE3) containing pET-32a-gD; 4, IPTG induced *E. coli* Rosetta (DE3) containing pET-32a; B: M and 1, *E. coli* Rosetta (DE3) containing pET-32a-gD before induction; 2, purified *E. coli* Rosetta (DE3) containing pET-32a-gD; C: 1, *E. coli* Rosetta (DE3) containing pET-32a-gD before induction; 2, Purified *E. coli* Rosetta (DE3) containing pET-32a-gD.

Figure 7. Antibody Titer of Mice Antiserum With Indirect Enzyme Linked Immunosorbent Assay



Mouse antiserum was diluted sequentially. 1, 1: 2000; 2, 1: 4000; 3, 1: 8000; 4, 1: 16,000; 5, 1: 32,000; 6, 1: 64,000; 7, 1: 128,000; 8, 1: 256,000; 9, 1: 512,000.

Figure 8. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of Purified mAbs (4E3, 3E7, 3F8, 1H3 and 4B6)



M, protein marker; 1-5, primary purified mAbs 3E7, 3F8, 4B6, 4E3 and 1H3.

## References

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