

## Expression of a Recombinant Protein Which Can Be Recognized by Antibody to *Toxoplasma gondii* SAG1

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### ABSTRACT

*Toxoplasma gondii* (*T. gondii*) infection can cause serious consequences in both humans and animals. Toxoplasmosis is a widespread zoonotic disease and thus is of economic as well as public health importance. Because toxoplasmosis lacks specific clinical signs or syndromes, the application of diagnostic techniques is required to detect the infection. Detection of antibodies to *T. gondii* has been very useful as an indicator of infection. Nevertheless, no final diagnosis will be made unless a native 30-kilodalton *T. gondii*-specific surface antigen 1 (SAG1) is recognized by the test samples. SAG1 is highly conserved among various strains of *T. gondii* and thus is a very useful molecule for diagnosis of toxoplasmosis. In this study, SAG1 gene was amplified and further purified as a 1,011-base pair nucleotide which was then ligated with pET-24b vector as a 6,320-base pair recombinant pET-24b/SAG1. After transformation, a 63-kilodalton protein was expressed in calcium chloride-treated competent *Escherichia coli*. This recombinant protein could be identified by *T. gondii*-infected cat sera which also were able to recognize SAG1 molecule by immunoblotting. Furthermore, all 30 sera which could recognize expressed recombinant protein were also positive in kinetics-based enzyme linked immunosorbent assay (ELISA) with recombinant protein. Therefore, ELISA with this expressed recombinant protein, combining the advantages of both traditional ELISA and time consuming immunoblotting, will be a powerful tool for surveying a large number of serum samples with excellent sensitivity and specificity. The potential candidate of this recombinant protein for vaccine development is also discussed.

**Key words:** *T. gondii*, recombinant protein, antibodies, SAG1, P30

### Introduction

*Toxoplasma gondii* (*T. gondii*) is an obligate intracellular Apicomplexa protozoan capable of infecting almost any warm-blooded vertebrates. Toxoplasmosis is a widespread zoonotic disease and thus is of economic as well as public health importance. Humans and animals become infected either by eating food contaminated with sporulated oocysts, or by consuming raw or undercooked meat containing cysts. *T. gondii* can cause death in infected neonatal and perinatal animals. In humans, congenital toxoplasmosis and fatal disseminated toxoplasmosis in acquired immunodeficiency syndrome patients are significantly important (Dubey, 1994; Davidson, 2000). Antibody detection has been very useful as an indicator of *T. gondii* infection (Lin and Bowman, 1991; Lin *et al.*, 1992). However, no final diagnosis can be made unless a

30 kilodalton (kD)-surface antigen 1 (SAG1 or P30) is recognized by the test samples (Gross *et al.*, 1992; Kasper and Khan, 1993).

SAG1 is an immunodominant *T. gondii* tachyzoite surface protein (Radke *et al.*, 2004) which also is an essential element for the attachment of host cells by the organism (Mineo and Kasper, 1994). SAG1 is a very useful marker molecule for differential diagnosis of *T. gondii* and closely related protozoan, *Neospora caninum* (Kimbita *et al.*, 2001; Mineo *et al.*, 2001). Sequence comparison has indicated that SAG1 is highly conserved through evolution despite parasite spreading world-wide (Hartati *et al.*, 2006). It has been shown that SAG1 can be recognized by antibodies against various strains of *T. gondii* (Kato *et al.*, 2007). We also showed that SAG1 could be recognized by sera from *T. gondii*-infected humans, cats, and dogs in Taiwan by immunoblotting (Lin,

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1998; Lin *et al.*, 1998; Lin *et al.*, 2004). Thus, SAG1 is a very useful molecule for serodiagnosis of toxoplasmosis. Although immunoblotting assay is able to detect the presence of antibody to SAG1, this technique is time consuming which combines electrophoresis of antigens under denaturing condition, an electrotransfer, and a specific antibody assay.

Enzyme-linked immunosorbent assay (ELISA) for serodiagnosis has the advantage of screening a large number of samples (Lin, 1998). Traditionally, soluble whole organism extracts are used as coating antigens for ELISA of which the specificity will be greatly enhanced if SAG1 is instead used. In this study, the gene encoding SAG1 was amplified by polymerase chain reaction. The amplified SAG1 deoxyribonucleic acid (DNA) was then cloned into prokaryotic expression vector pET-24b and subsequently expressed in *Escherichia coli* (*E. coli*). The expressed recombinant protein was further identified by SAG1-positive cat sera by immunoblotting and thereafter evaluated in kinetics-based ELISA.

## Materials and Methods

### *DNA extraction of T. gondii*

After proliferation in mouse peritoneal cavity, *T. gondii* RH strain tachyzoites were harvested by peritoneal lavage and washed with phosphate-buffered saline. They were further purified by 3  $\mu$ m Nuclepore polycarbonate membranes (Costar Corporation, Cambridge, MA). Purified *T. gondii* RH strain tachyzoites were lysed in buffer containing 0.5% sodium dodecyl sulfate, 100 mM sodium chloride, 10 mM ethylene diamine tetra-acetic acid, 10 mM Tris-chloride (pH 8.0), and 0.2 mg/ml proteinase K (Sigma Chemical Co., St. Louis, MO) at 55°C for 4 hr. Ribonuclease was then added to the final concentration of 100  $\mu$ g/ml and incubated at 55°C for 1 hr. DNA was extracted with phenol-chloroform, then precipitated with 3M sodium acetate at -20°C and pelleted by centrifugation at 10,000 xg for 10 min. The DNA pellet was washed in 70% ethanol and resuspended in TE buffer [10 mM Tris-chloride, 1mM ethylene diamine tetra-acetic acid (pH 8.0)]. The DNA was re-extracted and precipitated as above. The pellet was resuspended in TE buffer and DNA was quantified by measuring ultraviolet absorption at

260/280 nm. The extracted DNA was then amplified by polymerase chain reaction (Burg *et al.*, 1988).

### *Polymerase chain reaction*

Amplification of DNA was carried out in a 100- $\mu$ l reaction mixture containing reaction buffer (2 mM magnesium chloride, 250  $\mu$ M each of dATP, dGTP, dCTP, dTTP, 50 pmol of each primer, and 2.5 units of Taq DNA polymerase). All reagents were purchased from Promega Co., Madison, WI. The oligonucleotide primer set (ToEF-ToXR) used was designed according to the open reading frame of SAG1 gene which was published in Genebank S73634 (ZS strain, Chinese isolate). The sequences of the primers were: ToEF: 5'- CGG AAT TCG ATG TCG GTT TCG CTG CAC -3', and ToXR: 5'- CCG CTC GAG TCA CGC GAC ACA AGC TGA -3'. Both primers had the size of 27 base pairs (bp). Amplification was performed in a gene cycler (Bio-Rad Lab., Richmond, CA) over 30 cycles. Each cycle consisted of 1 min of denaturation at 94°C, 1 min of annealing at 50°C, and 1.5 min of extension at 72°C. Finally, an additional cycle at 72°C for 7 min was run. The products were then used for agarose gel electrophoresis and DNA recovery.

### *Agarose gel electrophoresis and DNA recovery*

Amplified polymerase chain reaction products (2-10  $\mu$ l) were analyzed with molecular size markers (Promega Co.) on a 1.2% MetaPhor agarose gel (FMC BioProducts, Rockland, ME) in Tris-acetic-ethylene diamine tetra-acetic acid buffer using a mini-horizontal gel electrophoresis apparatus (Bio-Rad Lab.). After electrophoresis, gel was stained with 1  $\mu$ g/ml ethidium bromide solution (Promega Co.) for 40 min and de-stained with two 30 min-washes of distilled water. The band was visualized by ultraviolet transillumination.

DNA recovery from agarose gel was performed with Quantum Prep Gel Slice Kit (Bio-Rad Lab.) according to manufacturer's instruction. Briefly, gel slices at which SAG1 gene DNA band remains were cut out using a clean razor blade. After chopped into pieces, they were transferred to spin column and frozen at -20°C for 5 min. The spin column was then spun at 12,000 xg for 1 min to recover purified DNA.

#### *Ligation of the vector pET-24b and SAG1 gene fragment*

Purified SAG1 gene DNA fragment was digested with restriction enzymes, EcoR I and Xho I. Reaction mixture was prepared by mixing 25 µl of purified DNA fragment, 3 µl of 10X EcoR I buffer, 1 µl of 20 U/µl EcoR I, 1 µl of 20 U/µl Xho I, and 1 µl of double distilled water. The mixture was mixed gently and then incubated at 37°C for at least 1 hr. Restriction enzyme activity was then heat inactivated at 65°C for 10 min. Vector pET-24b was digested with restriction enzymes EcoR I and Xho I. Reaction mixture was prepared by 20 µg of pET-24b DNA, 5 µl of 10X EcoR I buffer, 1 µl of 20 U/µl EcoR I, 1 µl of 20 U/µl Xho I, and a total volume of 50 µl was adjusted with double distilled water. After incubation at 37°C for at least 1 hr for complete digestion, the enzyme activity was heat inactivated at 65°C for 10 min. Linearized pET-24b and SAG1 gene fragments were mixed at a molar ratio of 1:3. One µl of 400 U/µl T4 DNA ligase and 1 µl of 10X ligase buffer were added and total volume was adjusted to 10 µl with double distilled water. Reaction mixture was incubated at 20°C for 3 hr, and terminated by heating at 65°C for 10 min. Ligation products were then digested with restriction enzymes and analyzed by 1.2% agarose electrophoresis. All reagents were purchased from Promega Co.

#### *Transformation of pET-24b/SAG1 into competent E. coli*

*E. coli* BL21(DE3) was streak from glycerol stock onto a Luria Bertani (LB) medium plate and grew overnight for 16-20 hr at 37°C. A single colony was picked and inoculated into LB broth which was then incubated at 37°C for 3-4 hr with shaking. When optical density at 600 nm reached 0.4-0.5, 20 ml of bacterial culture were transferred to a pre-chilled, sterilized 50-ml tube. After incubation on ice for 15 min, cell pellet was obtained by centrifugation at 4,500 xg for 5 min at 4°C. Cells were then resuspended gently in 7 ml of ice-cold 0.1 M calcium-chloride (CaCl<sub>2</sub>) and left on ice for 15-60 min. After centrifugation at 1,000 xg for 15 min at 4°C, bacteria were resuspended in 800 µl of ice-cold 0.1 M CaCl<sub>2</sub> and ready for transformation with pET-24b/SAG1 (Petersen *et al.*, 1998).

Pre-chilled 10 µl of recombinant pET-24b/SAG1 were mixed gently with 200 µl of

CaCl<sub>2</sub>-treated competent *E. coli* suspension and the mixture was placed on ice for 40 min. Supercoiled parental vector pET-24b was used as control. Transformation mixture was then heated at 42°C water bath for 90 sec and chilled on ice for 2 min. Eight hundred µl of SOC medium (Sigma Chemical Co.) were added into the mixture which was further incubated at 37°C for 1 hr with shaking to allow bacteria to express kanamycin resistance marker encoded by the plasmid. Transformed competent cells were obtained by centrifugation and further resuspended in 200 µl of SOC medium. Then, transformants were spread onto LBG/kanamycin plate (LB containing 20 mM glucose and 50 µg/ml kanamycin). Agar plates were left at room temperature until the liquid was absorbed, inverted and incubated at 37°C for 16-20 hr to obtain single colony of kanamycin resistant transformant.

#### *Expression of pET-24b/SAG1 recombinant protein*

A colony from the LBG/kanamycin plate was then transferred to 100 ml LBG broth and incubated at 37°C for 14 hr on a shaker. The culture was then diluted 1:100 with fresh pre-warmed LBG broth and further incubated at 37°C with shaking until optical density value at 600 nm reached 0.6. Isopropyl β-D-thiogalactopyranoside (Sigma Chemical Co.) was added to the final concentration of 1.0 mM to induce the expression of the recombinant protein and the culture was incubated for additional 4 hr. Bacteria were disrupted by repeated freezing-thawing and ultra-sonication and resulting supernatants were collected by centrifugation. After determination of the concentration by DC protein assay (Bio-Rad Lab.), expressed recombinant protein was subjected to immunoblotting analysis.

#### *Preparation of soluble T. gondii tachyzoite membrane proteins*

Purified *T. gondii* RH strain tachyzoites were suspended in 1% Nonidet-P40 (Sigma Chemical Co.)-50 mM Tris (pH 8.0). Following incubation at 4°C for 12 hr, the suspension was centrifuged and the resultant supernatant was collected as soluble membrane proteins. After determination of concentrations by DC protein assay (Bio-Rad Lab.), soluble membrane proteins were used in immunoblotting assay for the identification of SAG1.

#### *Immunoblotting assay for the identification of SAG1*

A minigel apparatus (Biometra Inc., Tampa, FL) was utilized for sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Soluble tachyzoite membrane antigens were mixed with reducing sample buffer (5% of 2-mercaptoethanol in sample buffer) and heated for 4 min at 90°C. Five µl of the above buffer, containing 25 µg of proteins or pre-stained molecular weight standards (Bio-Rad Lab.), were then applied to 12% sodium dodecyl sulphate-polyacrylamide gel and electrophoresed at 120 volts for 1 hr. The gel was then transblotted onto nitrocellulose paper (BA83, Schleicher & Schuell, Germany) by using a blotting apparatus (Biometra Inc.). After incubation with blocking solution [5% skim milk (Difco Lab., Detroit, MI) in phosphate-buffered saline] overnight at 4°C, nitrocellulose paper was incubated with 1:100 cat serum diluted in blocking solution with 1% Triton X-100 (Sigma Chemical Co.) at room temperature for 1 hr. The nitrocellulose paper was then washed and incubated with 1:5,000 peroxidase-labeled goat anti-cat immunoglobulin (Ig) G (Biogenesis Ltd., England, UK) at room temperature for an additional 1 hr. Once again, the nitrocellulose paper was washed and substrate containing 4-chloro-1-naphthol (Sigma Chemical Co.) was added. The positive result was visualized by the appearance of 30 kD SAG1 band. Thirty SAG1-positive and 30 negative cat serum samples were used for the following immunoblotting analysis of the expressed recombinant protein.

#### *Immunoblotting analysis of the expressed recombinant protein*

Thirty SAG1-positive and 30 negative cat blood serum samples were examined. Instead, 50 µg of proteins expressed by *E. coli* transformed with pET-24b/SAG1 plasmid were mixed with reducing sample buffer. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis, proteins were transferred onto nitrocellulose paper. Again, after addition of cat sera, peroxidase-labeled goat anti-cat IgG conjugate and substrate diaminobenzidine (Sigma Chemical Co.), the bands were visualized. Proteins obtained from *E. coli* containing pET-24b plasmid only were also used as control.

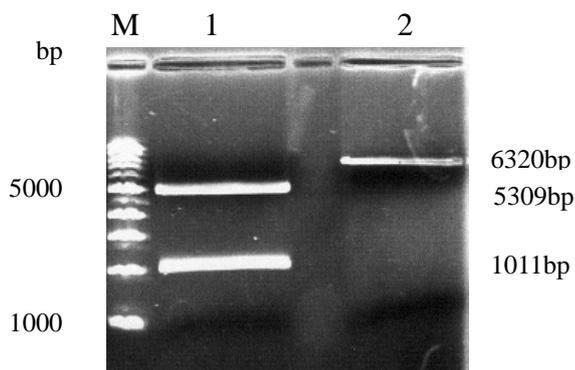
#### *Kinetics-based enzyme-linked immunosorbent assay with recombinant protein*

Fifty µl of expressed proteins (50 µg/ml in 0.1 M bicarbonate buffer, pH 9.6) were placed in each well of the MaxiSorp™ microtiter plates (Nunc, Roskilde, Denmark). The plate was incubated overnight at 4°C and washed with phosphate-buffered saline containing 0.05% Tween 20 by an ELISA washer (Dynatech Lab., Chantilly, VA). One hundred µl of phosphate-buffered saline containing 0.05% Tween 20 and 3% skim milk were then added as a blocking solution. After 40 min of incubation at 37°C, the plate was washed again. Fifty µl of 1:20 diluted cat serum in blocking solution were then added to the wells, followed by incubation at 37°C for 40 min. Thirty SAG1-positive and 30 negative cat blood serum samples were examined. After wash, 100 µl of 1:8,000 peroxidase-labeled goat anti-cat IgG Fc conjugate (Biogenesis Ltd.) in blocking solution were added. The plate was then incubated at 37°C for 40 min, washed, and finally rinsed with distilled water. One hundred µl of the substrate containing *O*-phenylenediamine (Sigma Chemical Co.) were added. The plate was read at 450 nm using a microplate reader (Dynatech Lab.). Antibodies specific for recombinant protein were detected by kinetics-based ELISA (Lin *et al.*, 1990). Accordingly, the rate of reaction between the bound peroxidase conjugate and substrate was determined by recording three absorbance readings at 2-min intervals each. These intervals provide a linear relationship between absorbance values and times so that the resulting sample regression coefficient (slope representing the rate of substrate conversion by enzyme) is directly proportional to the quantity of analyte present in the sample. This system precludes sources of error that go undetected in traditional ELISA and produces standard curves which allow normalization of data based on multiple control sera. Sera were screened in triplicate and the mean kinetics-based ELISA value of  $\geq 0.02$  optical density/sec was considered positive (Lin *et al.*, 1990).

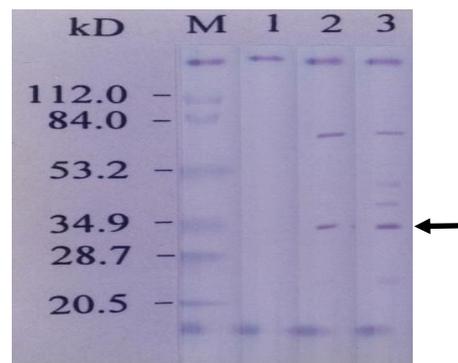
## **Results**

By using oligonucleotide primer set (ToEF-ToXR), the SAG1 gene fragment of *T. gondii* was amplified by polymerase chain reaction. After ethidium bromide staining on agarose gel, the

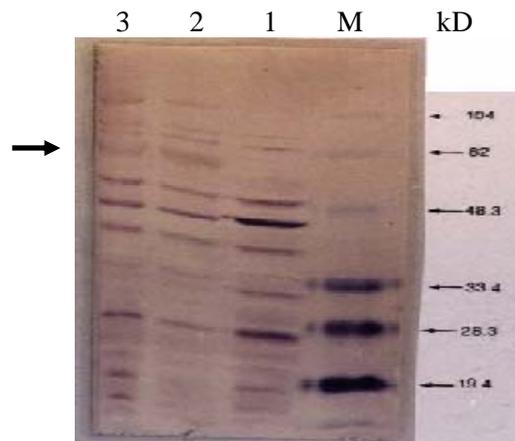
product appeared to be 1,011 bp in length (data not shown). After restriction enzyme digestion, purified SAG1 gene DNA fragment was ligated with linearized pET-24b. Agarose gel electrophoresis of recombinant pET-24b/SAG1 plasmid showed that ligation product had an approximate molecular size of 6,320 bp and linearized pET-24b vector only was 5,309 bp in length (Figure 1). Recombinant pET-24b/SAG1 was then transformed into CaCl<sub>2</sub>-treated competent *E. coli* cells. Only successfully transformed bacteria expressed kanamycin resistance marker encoded by the plasmid. *E. coli* containing pET-24b/SAG1 plasmid was induced by isopropyl β-D-thiogalactopyranoside to express recombinant protein. After sodium dodecyl sulfate polyacrylamide gel electrophoresis, proteins were transferred onto a nitrocellulose paper. After addition of SAG1-positive sera from *T. gondii*-infected cats (Figure 2), peroxidase-labeled anti-cat IgG conjugate and substrate, the recombinant protein was visualized as a 63 kD band (Figure 3). All 30 blood serum samples from *T. gondii*-infected cats which recognized SAG1 also were able to identify this recombinant protein. As a control, *E. coli* containing pET-24b plasmid only did not express this protein. In addition, all 30 sera which could recognize expressed recombinant protein were also found positive in kinetics-based ELISA with this recombinant protein (mean kinetics-based ELISA value ± standard deviation = 0.082 ± 0.009 optical density/sec). On the contrary, 30 uninfected cat sera did not have any positive reactions in both immunoblotting and ELISA (mean



**Figure 1.** Agarose gel electrophoresis of recombinant plasmid pET-24b/SAG1. Lane 1: EcoR I - Xho I restriction enzyme digestion of recombinant plasmid pET-24b/SAG1 showing linearized pET-24b vector (5,309bp) and SAG1 gene fragment (1,011bp); Lane 2: recombinant pET-24b/SAG1 plasmid (6,320bp); M: molecular size markers; bp: base pairs.



**Figure 2.** Immunoblotting assay for the identification of SAG1. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis, soluble *T. gondii* tachyzoite membrane antigens were transferred onto nitrocellulose paper. After addition of cat sera, peroxidase-labeled anti-cat IgG conjugate and 4-chloro-1-naphthol, the positive result was visualized by the appearance of 30 kD band (arrow). Lane 1: uninfected cat serum; Lanes 2&3: *T. gondii*-infected cat sera; M: pre-stained molecular weight standards; kD: kilodaltons.



**Figure 3.** Immunoblotting analysis of the expressed recombinant protein. *E. coli* containing pET-24b/SAG1 plasmid was induced by isopropyl β-D-thiogalactopyranoside to express recombinant protein. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis, proteins were transferred onto nitrocellulose paper. After addition of cat sera, peroxidase-labeled anti-cat IgG conjugate and diaminobenzidine, a 63 kD recombinant protein (arrow) was identified by SAG1-positive cat sera. Lane 1: proteins obtained from *E. coli* containing pET-24b plasmid and reacted with SAG-1 positive cat serum #1; Lane 2: proteins obtained from *E. coli* containing recombinant pET-24b/SAG1 and reacted with SAG1-positive cat serum #1; Lane 3: proteins obtained from *E. coli* containing recombinant pET-24b/SAG1 and reacted with SAG1-positive cat serum #2; M: pre-stained molecular weight standards; kD: kilodaltons.

kinetics-based ELISA value  $\pm$  standard deviation =  $0.006 \pm 0.001$  optical density/sec). Current data suggested that the both sensitivity and specificity for kinetics-based ELISA with this recombinant protein were 100%.

## Discussion

*T. gondii* infection can cause serious consequences in both humans and animals (Dubey, 1994; Davidson, 2000). Because toxoplasmosis lacks specific clinical signs or syndromes (Dubey, 1993), the application of diagnostic techniques is required to detect the infection. Mouse inoculation is sensitive and specific but time-consuming, taking up to 6 weeks to obtain a diagnosis (Derouin *et al.*, 1987; James *et al.*, 1996). Cell culture is the most practical method for the detection of *T. gondii* parasitemia, but this is also relatively slow and may lack sensitivity (Derouin *et al.*, 1987; James *et al.*, 1996). Polymerase chain reaction has been found to be a sensitive, specific and rapid method for the detection of *T. gondii* nucleic acid (James *et al.*, 1996; Jones *et al.*, 2000; Cresti *et al.*, 2001; Buchbinder *et al.*, 2003).

Nevertheless, for screening a large number of samples, antibody detection by ELISA is one of the best choices (Lin, 1998). In addition, by analysis of the amount of different classes of antibodies, ELISA even can supply us the information of infection status. IgG antibody is detectable at about 2 weeks after the infection (Lin and Bowman, 1991; Lin *et al.*, 1992), and remains measurable throughout the life of the host (Remington and Desmonts, 1983). Thus, detection of IgG antibody in a single sample cannot demonstrate the presence of active infection. Testing for IgM antibody (which appears at about 1 week after the infection) permits the detection of acute infection (Lin and Bowman, 1991; Lin *et al.*, 1992). Recently, by using IgG avidity ELISA based either on SAG1 or on recombinant SAG1, one can easily differentiate acute and chronic *T. gondii* infection (Sager *et al.*, 2003; Pietkiewicz *et al.*, 2007). The precise diagnosis of an acute and recent *T. gondii* infection in pregnant women and the newborn child is important before treatment. An early diagnosis of congenital toxoplasmosis can be made by detection of anti-SAG1 IgA antibodies (Decoster *et al.*, 1991) or by two-dimensional immunoblot differentiation of mother and child IgG profiles (Nielsen *et al.*,

2005).

Nonetheless, no final diagnosis can be made unless *T. gondii*-specific SAG1 is recognized by the test samples (Gross *et al.*, 1992; Kasper and Khan, 1993). SAG1 is the prototypic member of a superfamily of surface antigens called SAG1-related sequence. It constitutes the most abundant and predominant antigen on the surface of tachyzoites (Radke *et al.*, 2004). That is, for the purpose of enhancing specificity or the confirmation of toxoplasmosis, the traditional ELISA coating antigens should be replaced by SAG1. Luckily, SAG1 is highly conserved among various strains of *T. gondii* (Hartati *et al.*, 2006; Kato *et al.*, 2007) and can be recognized by sera from cats, dogs, and humans with toxoplasmosis in Taiwan by immunoblotting (Lin, 1998; Lin *et al.*, 1998; Lin *et al.*, 2004). Although immunoblotting is able to test SAG1 recognition of the sera, this technique is time consuming and is not suitable for survey purpose.

The gene encoding SAG1 of *T. gondii* has been cloned into the plasmid and subsequently expressed in *E. coli* as a glutathione-S-transferase fusion protein (Kimbata *et al.*, 2001). In our study, SAG1 gene was ligated with pET-24b vector as a recombinant pET-24b/SAG1 plasmid. After transformation, a 63 kD recombinant protein was expressed in *E. coli*. However, the molecular weight of this recombinant protein was larger than we expected. It is possible that dimer was formed in some way though more evidence is required to support this hypothesis. This recombinant protein could be identified by SAG1-positive sera from *T. gondii*-infected cats by immunoblotting. In addition, all 30 sera which could recognize expressed recombinant protein were also found positive in kinetics-based ELISA with this expressed recombinant protein. On the contrary, 30 negative sera could not recognize recombinant protein and also were negative in kinetics-based ELISA with this expressed recombinant protein. These results suggest that the recombinant protein expressed in our system shares antigenic epitope with SAG1 and the kinetics-based ELISA with this recombinant protein has 100% sensitivity and 100% specificity for detecting *T. gondii* antibody in cat sera in Taiwan. Therefore, kinetics-based ELISA with this recombinant protein, combining the advantages of both traditional ELISA and time consuming immunoblotting, will be a powerful tool for

surveying *T. gondii* infection in Taiwan. Previous results showed that traditional ELISA using soluble whole tachyzoite extracts as coating antigens for detecting IgG antibodies to *T. gondii* in cat sera had 100% sensitivity and 95.8% specificity (Lin and Su, 1997).

Furthermore, vaccines are promising for the control of toxoplasmosis (McLeod *et al.*, 1991; Wastling *et al.*, 1994). It has been shown that vaccination with peptides from the carboxy-terminal positions of SAG1 protected mice against a lethal challenge (Siachoque *et al.*, 2006). Recombinant SAG1 was also offer protection in experimental animal models (Petersen *et al.*, 1998; Nielsen *et al.*, 1999; Flori *et al.*, 2006). DNA vaccination with SAG1 protected against adult acquired *T. gondii* infection (Couper *et al.*, 2003). In vitro, the immunogenicity of SAG1 was notable based on the level of T-cell activation (Godard *et al.*, 1994), and synthesis of interferon- $\gamma$  and interleukin-12 suggested the participation of Th1 cells (Kato *et al.*, 2005). Consequently, the *T. gondii* recombinant protein developed in this study will also be an excellent potential candidate for vaccine development.

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## 表現一可被抗弓蟲 SAG1 抗體辨識之重組蛋白質

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### 摘 要

弓蟲感染在人跟動物都會造成嚴重的後果。弓蟲症是一分佈很廣的人畜共通傳染病，因此在經濟及公共衛生上皆很重要。由於弓蟲症缺乏特殊的臨床症狀，必須要使用診斷技術偵測感染。偵測抗弓蟲抗體，是一非常有用的感染指標。然而，只當測試樣品能辨識弓蟲獨特的表面抗原 1 (SAG1) 時，方能確定診斷。SAG1 在各種不同株的弓蟲其結構幾乎完全一樣，因此在弓蟲症診斷上為一非常有用的分子。在本研究，SAG1 基因經增幅並進一步純化出具有 1,011 base pairs 之核苷酸，然後連接上已去磷酸化直線形的 pET-24b 載體，形成具有 6,320 base pairs 之 pET-24b/SAG1 重組體。將此重組體植入氯化鈣處理過之 *Escherichia coli* 後，表現一 63 kilodaltons 之蛋白質。利用 immunoblotting 證實，此重組蛋白質亦可被能辨識 SAG1 分子之弓蟲感染貓的血清所結合。更進一步測試發現，所有 30 個能辨識表現的重組蛋白質之陽性血清，在使用此重組蛋白質之 kinetics-based enzyme linked immunosorbent assay (ELISA) 亦呈陽性。因此，使用此表現的重組蛋白質之 ELISA 結合了傳統 ELISA 與費時的 immunoblotting 兩者之優點，具有非常好的靈敏性與特異性，將成為一調查大量血清樣品之非常有效的工具。此重組蛋白質之可能發展為疫苗使用亦在文中討論。

**關鍵詞：**弓蟲、重組蛋白質、抗體、SAG1、P30

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