# Expression and purification of recombinant serine protease domain of the complement system protein C1s

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## Introduction

The complement system plays critical role in host defense against pathogens and inflammation (1). Complement activation also supports physiological apoptotic cells (AC) clearance and its downstream immunosuppressive effects (2). The classical pathway of complement activation is a major player in both the innate and adaptive antimicrobial host defense and in the maintenance of immune tolerance (3), in which the complement is triggered by multi-molecular initiation of complex $C_1$s component resulting in opsono-phagocytosis of invading pathogens and removal of immune complexes from the circulation.

Deficiency in any component of the classical pathway is associated with high incidence of immunological diseases and recurrence of bacterial infections. $C_1$s deficiency is associated with a high risk of systemic lupus erythematosis (SLE) development (4).

## Aim

The current work aimed to clone, express and purify C1s serine protease domain in order to study its characteristics and define the role of the classical pathway of complement activation during different bacterial infections.

## Materials and Methods

1) PCR Amplification of C1s coding sequence from mouse liver cDNA using Phusion® High-Fidelity DNA Polymerase kit.
2) Purification of the amplified product (720 bp) from the agarose gel using QIAquick gel extraction kit.
3) Double Digestion of the purified amplicon and the expression vector pRSET- B (3000 bp) using *Bam*HI & *Xho*I, followed by ligation using T4-ligase enzyme and transformation into chemically competent *E. coli* TOP 10.
4) Transformation of the recombinant expression vector pRSET- B into the competent *E. coli* BL-21 (DE3) pLysS, followed by pilot expression of the recombinant protein. SDS-PAGE and Western Blot analysis were performed to verify the protein identity.
5) Purification of the recombinant C1s-sp protein.

## Results

### 1) PCR amplification of C1s coding sequence

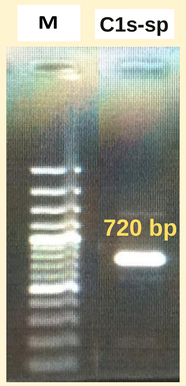


**Fig. (1): Agarose gel electrophoresis of C1s-sp coding sequence.**

### 2) Engineering of recombinant C1s-sp

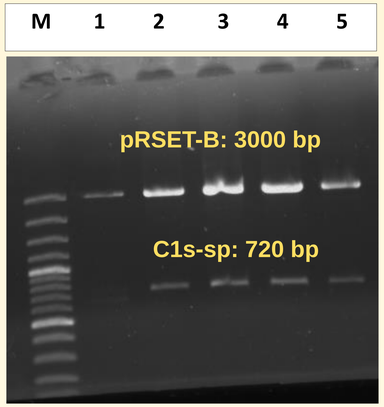


**Fig.(2): Agarose gel electrophoresis of the digested pRSET-B/C1s-sp recombinant vector.**

### 3) Sequence analysis

The alignment of C1s-sp coding sequence in pRSET-B was 100% identical with the corresponding reference sequence from GenBank.

### 4) Recombinant protein expression and purification

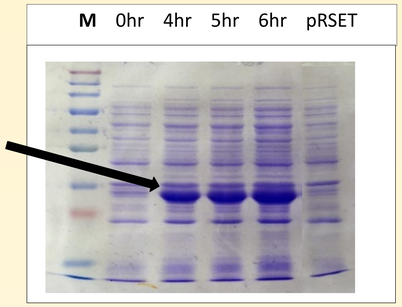


**Fig. (3): Time course expression of recombinant C1s-sp (32 kDa).**

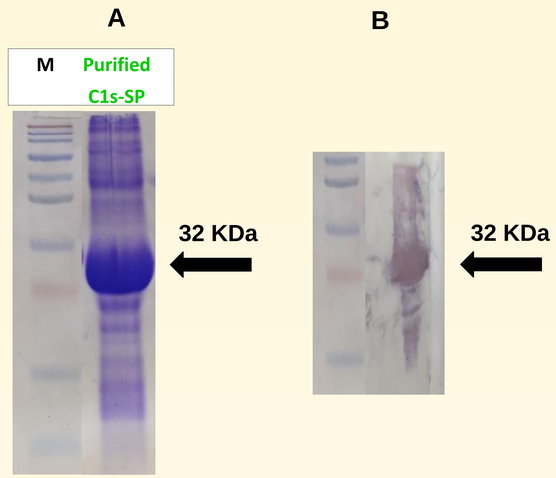


**Fig. (4): A- SDS-PAGE of purified C1s-sp protein (32 kD) stained with Coomassie blue. B- The protein identity was confirmed via Western blot using anti-histidine tag antibody.**

## Conclusion

- Recombinant DNA technology was successfully applied to construct C1s serine protease domain using bacterial expression systems.
- Expression of recombinant C1s serine protease domain was successfully achieved using BL-21 (DE3) pLysS with high levels of protein expression. The current study opens the door in order to study the characteristics of C1s, and define the role of the classical pathway of complement system activation during different bacterial infections.

## References

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