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Bioproduction and reconstruction of anti-C-reactive protein monoclonal antibody

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Introduction

C-reactive protein (CRP) is an annular pentameric protein synthesized by the liver and sent into the bloodstream in response to inflammation. Measuring the serum CRP level can provide useful information for determining disease progress [1]. Consequently, there is a large demand of high-quality CRP antibody for immunoassay, which can't be fulfilled by traditional method produced from hybridoma cells. Here, we developed a method to express recombinant anti-CRP antibody with high quantity and quality.

Objectives

- ❑ **Recombinant anti-CRP plasmids construction**
- ❑ **Recombinant anti-CRP antibody expression with high expression level.**
- ❑ **Recombinant anti-CRP antibody functional examination**

Methods

Using next-generation sequencing, the sequence information of anti-CRP antibody was obtained from a previously established anti-CRP antibody hybridoma cell line 7E12. The anti-CRP antibody plasmid was constructed with recombinant mouse IgG1 Fc region for heavy chain and Igκ for light chain respectively. After confirmation of the sequence accuracy, the plasmids were transiently transfected into ExpiCHO cells for anti-CRP antibody production. After affinity purification using protein G beads, the amount of the anti-CRP antibody was determined by BCA, and the binding activity of anti-CRP was measured by ELISA.

❑ Flowchart of anti-CRP antibody bioproduction

a. Culture of Anti-CRP antibody hybridoma Cell line 7E12 and total RNA extraction

b. Variable heavy chain and variable light chain DNA fragments amplification and purification

c. NGS sequencing and sequences conformation

d. Construction of anti-CRP antibody plasmids

e. Recombinant anti-CRP antibody expression and functional examination

a. The anti-CRP antibody hybridoma cell line was cultured, following by RNA extraction.

b. Using RT-PCR and PCR with high fidelity polymerase to amplify the variable regions of heavy chain and light chain separately.

c. The sequence information of purified DNA fragments was obtained by NGS sequencing and was confirmed via comparing with sequence from IMGT database.

d. The expression plasmids constructed on pcDNA3.1(+). The constant regions used were mouse IgG1 constant region (GenBank: AAK53870.1) and mouse Igκ constant region (Uniprot: A0A5H1ZRK8).

e. Recombinant anti-CRP antibody were expressed in ExpiCHO expression system, following by antibody purification and functional examination.

Reference:
[1] Nehring, S.M., et al., *C Reactive Protein (CRP)*, in *StatPearls*. 2020, StatPearls PublishingCopyright © 2020, StatPearls Publishing LLC.: Treasure Island (FL).

Results

❑ Recombinant anti-CRP plasmids construction.

A

Heavy Variable Fragment Alignment	
Regions	Alignment with top germline V gene
FR1	97.3 %
CDR1	95.8 %
FR2	96.1 %
CDR2	95.8 %
FR3	97.4 %
CDR3	100 %
FR4	96.9 %
TOTAL	96.9%

B

Light Variable Fragment Alignment	
Regions	Alignment with top germline V gene
FR1	98.7 %
CDR1	100 %
FR2	100 %
CDR2	100 %
FR3	100 %
CDR3	100 %
FR4	100 %
TOTAL	99.7%

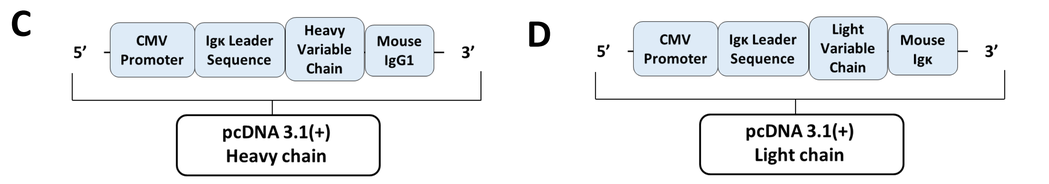

Figure 1. A and B: Heavy chain variable region and Light chain variable region sequences identification on IMGT database. C and D:The schematic diagram of the expression plasmid for heavy chain and Light Chain.

❑ Anti-CRP antibody expression in ExpiCHO system.

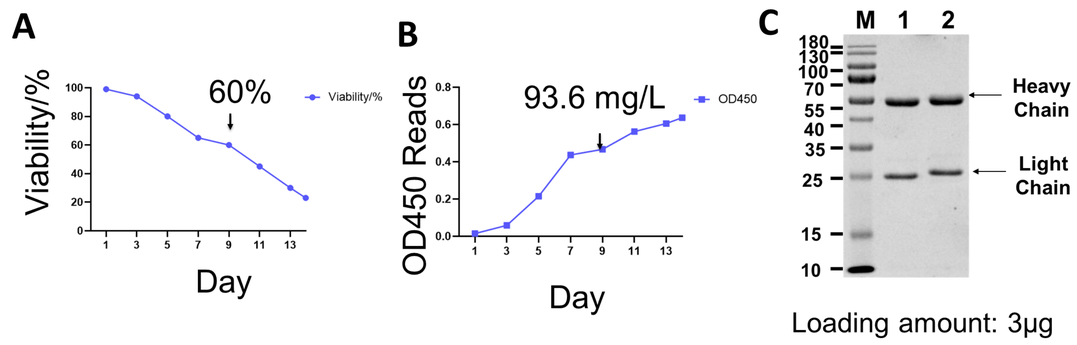

Figure 2. A. The dynamic monitoring of cell viability. Viability was determined every 2 days. B. The dynamic monitoring of Anti-CRP antibody level. The anti-CRP level was measured by every 2 days. Samples were diluted 5000 times for ELISA test. C. SDS-page analysis of purified recombinant anti-CRP antibody. lane 1: Anti-CRP Antibody derived from Hybridoma cell line 7E12; lane 2: Recombinant Anti-CRP Antibody derived from ExpiCHO cells.

❑ Recombinant anti-CRP antibody functional examination

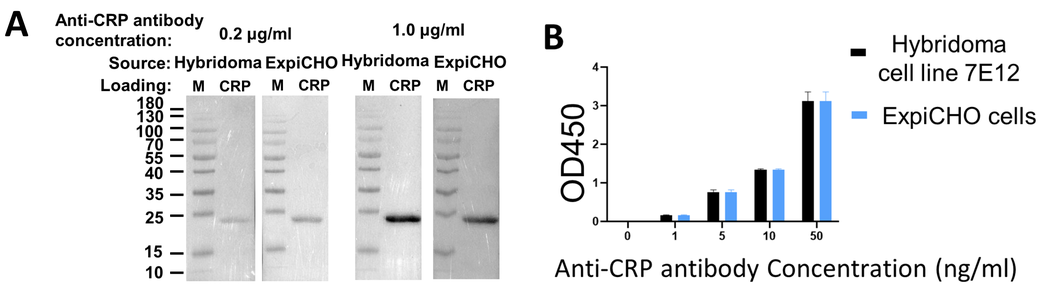

Figure 3. A. Western blot analysis for recombinant anti-CRP antibody. The recombinant anti-CRP antibody (source: ExpiCHO) was comparing with antibody secreted from hybridoma cell line 7E12 (source: Hybridoma), with the incubation concentration was 0.2 μg/ml and 1.0 μg/ml separately. B. Sensitivity test for recombinant anti-CRP antibody. ELISA test (shown in OD450 reads) were conducted for comparing the binding activity. The recombinant anti-CRP antibody (ExpiCHO cells) was comparing with antibody secreted from hybridoma cell line 7E12 (Hybridoma cell line 7E12) (n=3). The CRP coating amount was 2 μg/ml.

Conclusion

1. The recombinant anti-CRP antibody expression plasmids were successfully constructed based on variable region sequences obtained from hybridoma cell line.
2. Using ExpiCHO expression system, the expression yield of newly recombinant anti-CRP antibody was above 90 mg/L, which was much higher than that from hybridoma cell line (30-40 mg/L).
3. By comparing the performances of Western blot and ELISA, the functional activity of recombinant anti-CRP antibody was comparable to that of the anti-CRP antibody from hybridoma cell line.