



Expression and antibody generation of the cancer-testis antigen, BIOT2-S

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ABSTRACT. *Biot2-S* is a mouse cancer-testis antigen gene that was identified using the cross-reactive serological analysis of recombinant cDNA expression libraries (SEREX) technique in the State Key Laboratory of Biotherapy, West China Hospital, Sichuan University. To express BIOT2-S and generate its antibody for further investigation, the *Biot2-S* prokaryotic recombinant expression vector Biot2-S/pGEX6P-1 was constructed with *Escherichia coli* DH5 α as a cloning vector, and BIOT2-S was expressed in *E. coli* Rosetta (DE3). The recombinant BIOT2-S was expressed in the form of an inclusion body and the targeted recombinant BIOT2-S was produced at the level of approximately 25% total bacterial proteins after being induced with optimum conditions (0.2 mM isopropyl- β -D-thiogalactopyranoside for 6 h at 37°C). The target protein was purified by glutathione S-transferase (GST)-trap FF affinity chromatography and detected by western blot. The purified recombinant protein was further confirmed by electrospray ionization

quadrupole time-of-flight mass spectrometry after removal of the GST-tags. Then the purified BIOT2-S was used to immunize adult rabbits to generate its antibody. The antibody was purified and its specificity determined. The titer of the antibody was shown to reach 10^4 and the antibody was demonstrated to be able recognize the corresponding protein in the testes of mouse and chicken; the tumor cell lines CT-26 and S180 also reacted with the antibody. This study provides a valuable foundation for further research on the cancer-testis antigen BIOT2-S.

Key words: *Biot2-S*; Cancer-testis antigen gene; Polyclonal antibody; Recombinant expression