

## ELISA Based hTSH Assessment Using Two Sensitive and Specific Anti-hTSH Polyclonal Antibodies

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**Abstract :** Production of specific antibody responses against hTSH is a cumbersome process due to the high identity between the hTSH and the other members of the glycoprotein hormone family (FSH, LH and HCG) and the high identity between the human hTSH and host animals for antibody production. Therefore, two polyclonal antibodies were purified against two recombinant proteins. Four possible ELISA tests were designed based on these antibodies. These ELISA tests were checked against hTSH and other glycoprotein hormones, and their sensitivity and specificity were assessed. Bioinformatics tools were used to analyze the immunological properties. After the immunogen region selection from hTSH protein, c terminal of B hTSH was selected and applied. Two recombinant genes, with these cut pieces (first: two repeats of C terminal of B hTSH, second: tetanus toxin+B hTSH C terminal), were designed and sub-cloned into the pET32a expression vector. Standard methods were used for protein expression, purification, and verification. Thereafter, immunizations of the white New Zealand rabbits were performed and the serums of them were used for antibody titration, purification and characterization. Then, four ELISA tests based on two antibodies were employed to assess the hTSH and other glycoprotein hormones. The results of these assessments were compared with standard amounts. The obtained results indicated that the desired antigens were successfully designed, sub-cloned, expressed, confirmed and used for *in vivo* immunization. The raised antibodies were capable of specific and sensitive hTSH detection, while the cross reactivity with the other members of the glycoprotein hormone family was minimum. Among the four designed tests, the test in which the antibody against first protein was used as capture antibody, and the antibody against second protein was used as detector antibody did not show any hook effect up to 50 miu/l. Both proteins have the ability to induce highly sensitive and specific antibody responses against the hTSH. One of the antibody combinations of these antibodies has the highest sensitivity and specificity in hTSH detection.

**Keywords :** hTSH, bioinformatics, protein expression, cross reactivity

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