



Turkish Journal of Medical Sciences

Turkish Journal
of
Medical Sciences

Development of ELISA Systems for Measurement of Human Tumor Necrosis Factor-Alpha (TNF- α)

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Abstract: The aim of this study was to develop a human tumor necrosis factor alpha (hTNF- α) ELISA system because specific and sensitive measurement of low levels of circulating TNF-a is very important for enlightening the immunopathological mechanisms associated with TNF- α . Monoclonal antibodies 6A4c and 8A6 were produced against hTNF- α and were used as the capture antibody and the tracer antibody respectively for the first hTNF- α ELISA system (6A4c/biotin-8A6). Murine polyclonal IgG was used as the tracer antibody in the second hTNF- α ELISA system (6A4c/biotin-polyclonal IgG). Both systems could detect both recombinant hTNF- α and native hTNF- α . The detection limits defined as minimal concentration of hTNF- α were less than 4 pg/ml for the first and less than 12 pg/ml for the second ELISA systems. 6A4c/biotin-8A6 system resulted in some non-specific reactions to some extent with human sera; however, 6A4c/biotin-polyclonal IgG system produced acceptable background levels with human sera. A prominent inhibitory effect of TNF receptors-I and -II did not occur in any of the ELISA systems at physiological concentrations. Two different types of ELISA systems with high sensitivity and specificity were developed to measure hTNF- α level both in human serum and cell culture supernatant.

Key Words: TNF- α , ELISA

Turk J Med Sci 2005; **35**(6): 385-393.

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