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American Association for Cancer Research

TITLE (QPS 2010-001)

GLP validiation for quantitative determination of soluble tumor necrosis factor receptor I and II (sTNFRI and sTNFRII) in human serum utilizing a highly sensitive ELISA

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ABSTRACT

Introduction

TNFs are pleiotropic cytokines that play an essential role in host immune resistance to tumor formation and infections. All cell types examined expressed at least one of the two distinct TNF receptors I and II. The soluble receptor forms resulting from shedding of the extracellular domains of the receptors. The elevated sTNFRI and sTNFRII seems to be important biomarkers in association with malignancies and infections. The purpose of this study is to validate high sensitivity ELISA methods adapted from commercial kits (R&D Systems, Minneapolis, MN) for quantification of human sTNFRI or sTNFRII in human serum to support clinical studies of new drug development for inflammatory diseases, organ failures, and cancer therapies.

Methods

Both assays are a two-site sandwich ELISAs using two selected antibodies that bind to different epitopes of human sTNFRI or sTNFRII. The plates are pre-coated with target-specific antibody. Samples at a minimum required dilution of 1:10 are incubated and the immobilized antibody binds any analyte present. After washing away any unbound substances, an enzyme-conjugated antibody specific for sTNFRI or sTNFRII is added to the wells. The immobilized immunocomplex sandwich is detected by incubation with a substrate solution in a timed reaction. The color development is measured in a SpectraMax 384 Plus microplate reader. The enzymatic activity is directly proportional to the amount of sTNFRI or sTNFRII in the sample. The results were analyzed by a 4-parameter regression and sample concentrations are determined directly from a standard curve.

Results

The ELISA methods were validated over a range of 80 to 1500 pg/mL for sTNFRI and 40 to 1000 pg/mL for sTNFRII in human serum. Quality control samples at 5 concentrations were used to determine precision and accuracy. Intra-assay precision ranged from 1.7 to 8.1%, while inter-assay precision ranged from 3.7 to 8.9%. Intra-assay accuracy (defined as % error from nominal) ranged from -0.2 to -4.3% and the inter-assay accuracy ranged from -2.5 to 6.6%. We demonstrate that both analytes are stable in human serum for at least 17 hours at room temperature and after 5 freeze/thaw cycles at -20°C or -70°C. Both analytes are also stable in human serum for at least 60 days at -20°C or -70°C. The endogenous serum concentrations in the healthy subjects ranged from 2,448 to 7,139 pg/mL for sTNFRI and from 2,706 to 5,752 pg/mL for sTNFRII. The dilution reproducibility, matrix effect and recovery will also be presented.

Conclusion

Highly sensitive ELISA methods have been validated for the quantitation of sTNFRI and sTNFRII in human serum. The assays were proved to be sensitive, selective, accurate, reproducible and reliable as a bioanalytical method for assessing.