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ProductInformation

ANTI-HUMAN TUMOR NECROSIS FACTOR SOLUBLE RECEPTOR I (TNF sRI)
Developed in Goat, Affinity Isolated Antibody

Product Number **T 2065**

Product Description

Anti-Human Soluble Tumor Necrosis Factor Receptor I (sTNF RI) is developed in goat using a recombinant human sTNF RI, expressed in *E. coli* as immunogen. This recombinant protein represents the non-glycosylated, N-terminal methionyl form of the naturally occuring human soluble type I receptor for TNF. The antibody is purified using TNF RI affinity chromatography.

Anti-Human sTNF-RI may be used to detect human sTNF RI by immunoblotting and ELISA. By immunoblotting and ELISA, the antibody shows <10% cross-reactivity with recombinant mouse sTNF-RI. In addition, the antibody shows no cross-reactivity with other cytokines tested.* The antibody can interact with the cell surface human TNF RI (55 kDa) and exhibits TNF agonist activities on the human cell line A549 and the mouse cell line L929.

Monoclonal Anti-Human sTNF-RI may be used as an agonist for human cell surface TNF RI biological activity and for the detection of sTNF RI by immunoblotting and ELISA.

TNF RI (CD120a) is a 55 kDa transmembrane glycoprotein that is expressed by virtually all nucleated mammalian cells.[1-3] Among the numerous cells known to express TNF RI are hepatocytes,[4] monocytes and neutrophils,[5] cardiac muscle cells,[6] endothelial cells,[7] and $CD34^+$ hematopoietic progenitors.[8] Both TNF-α and TNF-β bind to TNF RI. Soluble TNF-α binds with a kDa in the range of 20-60 pM,[9,10] while TNF-β binds with a kDa equal to 650 pM.[9] TNF RI relative to TNF RII seems to be the more physiologically-relevant receptor, whereas TNF-R2 appears to play a direct role in only a limited number of TNF responses.[11,12] Soluble TNF RI, which blocks TNF-α activity, has been identified in both urine and blood (1-3 ng/mL).[4,13,14]

Serum levels of sTNF receptors increase dramatically in certain pathological situations. Soluble forms of two molecular weights (p60 and p80) have been identified and are believed to be generated by proteolytic cleavage.[3,15,-17]

Human TNF RI has 64% amino acid sequence identity (70% in the extracellular region) with mouseTNF-R1, and human TNF RI binds human and mouse TNF-α with equal affinity.[18,19]

The extracellular region has four cysteine-rich motifs, the first of which is suggested to be required for binding.[9] The intracellular portion of TNF R1 contains a "death domain" of about 70 amino acids that is required for the signaling of apoptosis and NF-κB activation.[20,21] TNF binds to the extracellular domain of TNF R1 and induces receptor trimerization.[22] Then, the aggregated death domain of TNF R1 recruits the adapter protein TRADD.[21] TRADD, in turn, recruits FADD, TRAF2 and RIP to form the TNF R1 signaling complex and activate signaling cascades leading to apoptosis,[23,24] JNK/SAPK activation,[23,25] and NF-κB activation[26,27] respectively. However, TNF R1 self-associates and signals independently of ligand when overexpressed. This apparent paradox may be explained by silencer of death domains (SODD), a widely expressed approximately 60 kDa protein that was found to be associated with the death domain of TNF-R1.[28]

Reagents

The product is supplied lyophilized from a 0.2 µm filtered solution in phosphate buffered saline. Endotoxin level is < 10 ng per mg antibody as determined by the LAL method.

Preparation Instructions

To one vial of lyophilized powder, add 1 ml of 0.2 µm-filtered PBS to produce a 0.1 mg/ml stock solution of antibody. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

Storage/Stability
Prior to reconstitution, store at –20 °C. Reconstituted product may be stored at 2-8 °C for at least one month. For prolonged storage, freeze in working aliquots at –20 °C. Avoid repeated freezing and thawing.

Procedure
Anti-Human sTNF RI is tested for its agonist activity in a cytotoxicity assay in the presence of the metabolic inhibitor, actinomycin D, using mouse L929 cells.[29]

Product Profile
For agonist activity, typically the ED_{50} for cytotoxicity of L929 cells is 0.5 – 2 µg/ml.

For Indirect Immunoblotting, a working concentration of 0.1 – 0.2 µg/ml is determined using recombinant human sTNF RI at 1 ng/lane under non-reducing conditions and 25 ng/lane under reducing conditions.

For Indirect ELISA, a working concentration of 0.5 - 1 µg/ml is determined to detect recombinant human sTNF RI to a limit of 1 ng/well.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilutions by titration test.

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*rhsTNF RII, rmsTNF RII, rhTNF-α, TNF-β

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