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Humankine® product line
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HUMANKINE® TNF ALPHA:

Authentic Trimer – High Efficiency Human Cell Expressed.

Introduction:

Cytokines are a group of proteins and polypeptides that organisms use as signaling molecules. Most cytokines are glycoproteins less than 30 kDa in size and bind to specific, high-affinity cell surface receptors. Due to their central role in the immune system, cytokines are involved in a variety of immunological, inflammatory and infectious diseases and widely used in research, diagnostics and therapeutics. Currently, these proteins are predominantly produced in non-human cells (e.g. E. coli, SF9, CHO) and therefore lack authenticity due to the absence of physiologically relevant glycosylation. In addition, a number of important cytokines are not commercially available due to inadequate proteolytic processing, protein folding or other posttranslational modifications that do not occur in the non-human cell expression systems. Proteintech has developed an efficient human-cell based technology for the scalable production of human cytokines.

TNF alpha :

TNF alpha is a member of the prototypic ligand of the TNF superfamily. This cytokine plays a central role in inflammation, apoptosis, and immune system development. The native 26 kD transmembrane protein is assembled intracellularly to form a noncovalently linked homotrimer[1]. Cleavage of the membrane bound TNF alpha by TACE/ADAM17 releases a 55 kD soluble trimeric form that regulates lymphoid tissue development and promotes inflammation responses[2].

Other commercial sources of TNF alpha proteins are produced from E. coli. Proteintech has produced Humankine TNF alpha from engineered human 293 cells. Both E. coli and human cell expressed proteins exhibit a molecular mass of 17 kD by SDS-PAGE. However, gel filtration studies indicate that only Humankine TNF alpha is trimeric, which has a molecular mass of 51 kD, while the E. coli form is not a trimer *(Figure 1)*. These proteins also show distinct mobility on native gel electrophoresis.

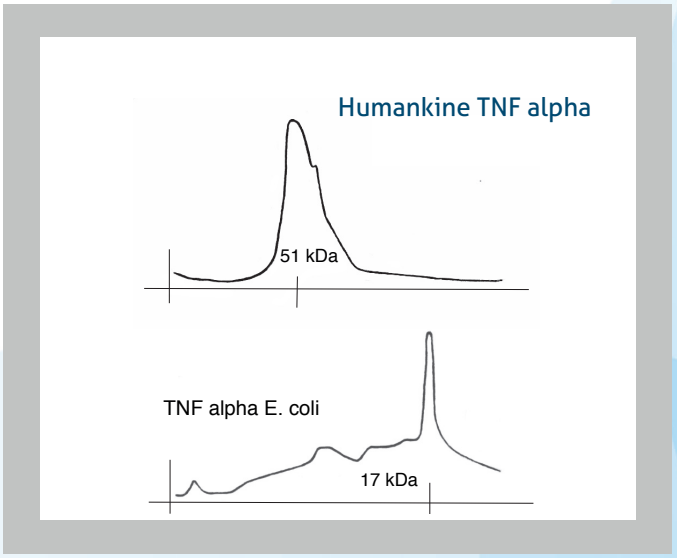

Figure 1 – Comparison of molecular weight of Humankine TNF alpha to E. coli expressed TNF alpha

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The bioactivity of Humankine TNF alpha was determined by the dose-dependent cytotoxicity of the TNF alpha sensitive cell line L-929 in the presence of Actinomycin D.

The biological effect was also compared with RA synoviocytes. 500,000 cells/well were used in 6 well plates. Cells were starved for 12h with DMEM media without FCS. Cells were then stimulated for 24h in 2 ml of DMEM without FCS. The IL-6 production was analyzed by ELISA on the supernatants. The data in the figure below indicate that Humankine TNF alpha is more effective than the E. coli expressed cytokine *(Figure 2)*.

Proteintech has developed and continues to develop a growing range of tag-free cytokines, including difficult-to-express protein members of the TGF beta I superfamily. Proteintech's cytokines are produced to be Xeno-free to address concerns caused by trace animal components or mammalian pathogens. All Humankine proteins are recombinant, animal component-free, and solely from human origin. There are no trace elements introduced as is commonly the case when exotic expression in E. coli, yeast and CHO is employed. Additionally, the internal machinery in human expression systems means our products will have bona-fide post-transcriptional modifications, such as phosphorylation and glycosylation, among others. Humankine cytokines can be used as highly preferred reagents in a wide range of applications for cancer, inflammation, stem cell research, and antibody development.

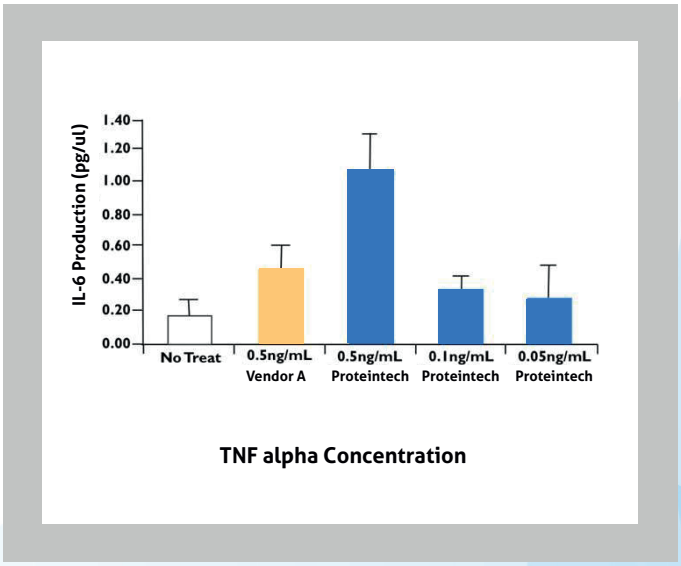

Figure 2 – Proteintech TNF alpha: Higher Efficacy-production of IL-6 in Rheumatoid Synoviocytes

References:

1. Tang, P et al., 1996 Biochemistry 35:8216
2. Black, R.A et al., 1997, Nature 385:729

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