

## TNF alpha<sup>HuXp</sup> Authentic Trimer - High Efficacy Human Cell Expressed



### INTRODUCTION

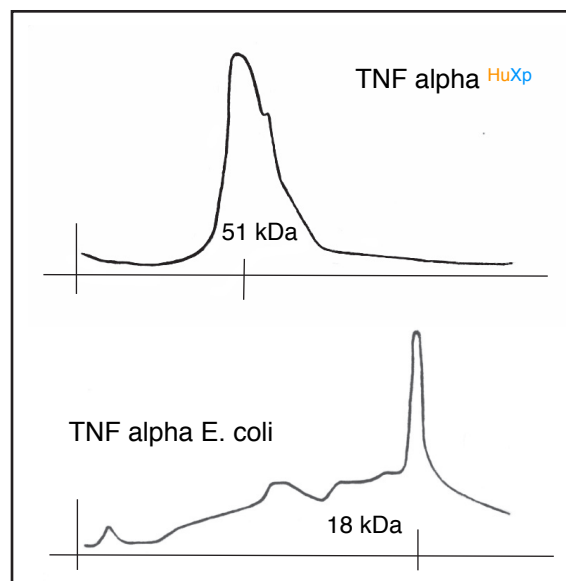
Cytokines are a group of proteins and polypeptides that organisms use as signaling molecules. Most cytokines are glycoproteins less than 30 kDa in size and bind to specific, high-affinity cell surface receptors. Due to their central role in the immune system, cytokines are involved in a variety of immunological, inflammatory and infectious diseases and widely used in research, diagnostics and therapeutics. Cytokines generally alter the gene expression pattern of the target cell which can lead to changes in the rate of cell proliferation and/or in the state of cell differentiation. 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 post-translational modifications that do not occur in the non-human cell expression systems. HumanZyme has developed an efficient human-cell based technology, HumaXpress™, for scalable production of human cytokines.

### TNF alpha<sup>HuXp</sup>

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<sup>1</sup>. 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<sup>2</sup>.

Other commercial sources of TNF alpha proteins are produced from *E. coli*. HumanZyme has

produced TNF alpha<sup>HuXp</sup> from engineered human 293 cells. Both *E. coli* and human cell expressed proteins exhibit a molecular mass of 18 kD by SDS-PAGE. However, gel filtration studies indicate that only TNF alpha<sup>HuXp</sup> is trimeric, which has a molecular mass of 51 kD, while the *E. coli* form is not a trimer. These proteins also show distinct mobility on native gel electrophoresis.



The bioactivity of TNF alpha<sup>HuXp</sup> was determined by the dose-dependent cytotoxicity of the TNF alpha sensitive cell line L-929 in the presence of Actinomycin D.

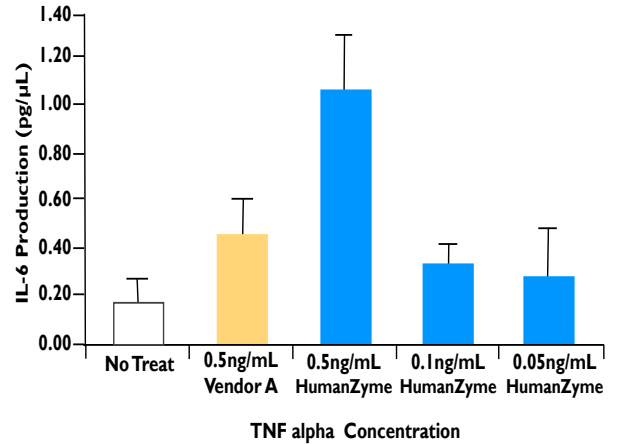
The biological effect was also compared with RA synoviocytes. 500,000 cells/wells were used in 6 well plates. Cells were starved for 12h with DMEM media without FCS. Cells were then stimulated for 24h in 2 ml of DMEM without FCS. The IL6 production was analyzed by ELISA on the supernatants. The data in the figure below indicate that TNF alpha<sup>HuXp</sup> is more effective than

the E coli-expressed cytokine.

HumanZyme 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. HumanZyme cytokines are produced to be Xeno-free to address concerns caused by trace animal components or mammalian pathogens. All HumaXpress™ products 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. HumanZyme Authentic Cytokines can be used as highly preferred reagents in a wide range of applications for cancer, inflammation, stem cell research, and antibody development.

#### References

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



TNF alpha<sup>HuXp</sup>: Higher Efficacy-Production of IL-6 in Rheumatoid Synoviocytes