

Authentic IL-23^{HuXp} Expressed in Human Cells 100x More Potent For Induction of Human TH17 Cells



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 post-translational modifications that occur in the non-human cell expression systems. HumanZyme has developed an efficient human-cell based technology, HumaXpress™ for the scalable production of human cytokines. The company is expanding this range of tag-free produced cytokines, including difficult-to-express members of the TGFβ superfamily. HumanZyme's authentic cytokines are preferred reagents for stem cell, cancer, inflammation research, and antibody development.

IL-23^{HuXp}

Currently, commercially available recombinant IL-23 cytokine is produced as a heterodimeric or fusion protein from an insect cell expression system. HumanZyme has produced IL-23^{HuXp} in a stable cell culture of engineered human HEK293 cells. The protein is expressed as a disulfide-linked dimer of 55 kD and, due to the scalability of the stable culture, can be cost-effectively produced. (Fig. 1)

IL-17-producing CD4⁺ T cells (Th17 cells) have been identified as a unique subset of T helper cells that develop along a pathway that is distinct from the Th1 and Th2-cell differentiation pathways. This finding has provided exciting new

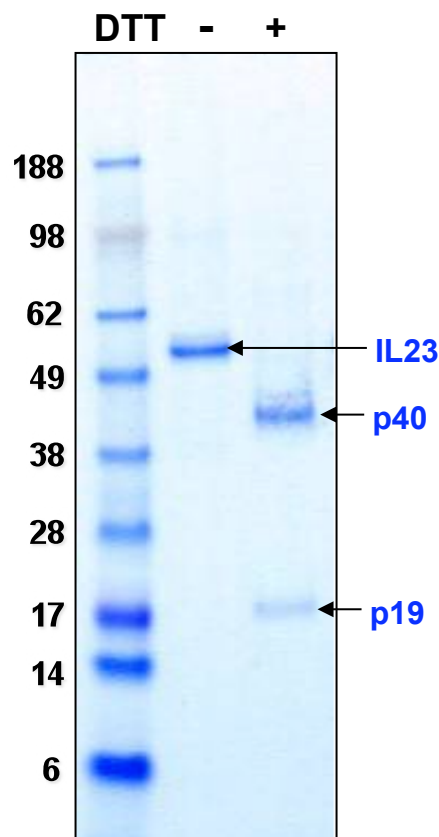


Fig. 1. Highly purified glycosylated, dimeric recombinant human IL-23^{HuXp} expressed in an HEK293 human cell expression system.

insights into immunoregulation, host defense and the pathogenesis of autoimmune diseases. Recently it has been shown that TGF-β1, IL-1β, IL-6 and IL-23 are important in driving human Th17 differentiation. The bioactivities of IL-23 from human and insect cells were first determined by the dose-dependent secretion of IL-17 from mouse splenocytes activated with 10 ng/ml PMA, which shows that IL-23^{HuXp} is ten fold more active. (Fig. 2). The activities were further assayed with human CD4⁺ cells which were isolated from a healthy donor and stimulated with 10 μg/ml plate bound anti-CD3 and 10 μg/

ml soluble anti-CD28 in the presence of Th17 polarizing cytokines. After 5 days supernatants were harvested for measurement of IL-17 by ELISA. The results show that IL-23^{HuXp} is 100-fold more potent for inducing IL-17 secretion in two independent studies, maximum induction was achieved with 0.1 ng/ml IL-23^{HuXp} vs 10ng/ml with insect cell-produced IL-23 (Fig. 3). These results demonstrate that authentic human cell expressed cytokines can induce Th17 cell differentiation at physiologically relevant concentration and may lead to more accurate scientific understanding of human biological process.

A rapidly expanding range of **HumaXpress™** cytokines are available from HumanZyme Inc. The proteins are manufactured to high quality standards and provide high biological activity, lot-to-lot consistency and low endotoxin levels. The specific products discussed here, IL-23^{HuXp}, TGF-β1^{HuXp}, IL-1 beta^{HuXp} and IL-6^{HuXp} are available in convenient pack sizes including trial size and bulk.

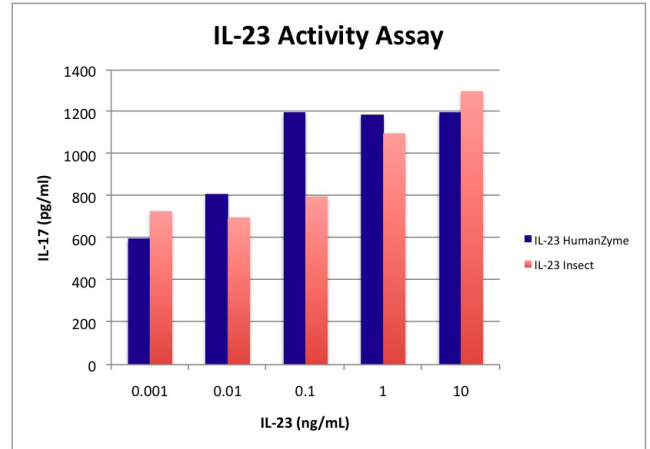


Fig. 3. IL-23 activities determined by the dose-dependent secretion of IL-17 from human CD4+ T cells stimulated with 2 mg/ml plate bound anti-CD3 and 1 mg/ml soluble anti-CD28 in the presence of Th17 polarizing cytokines.

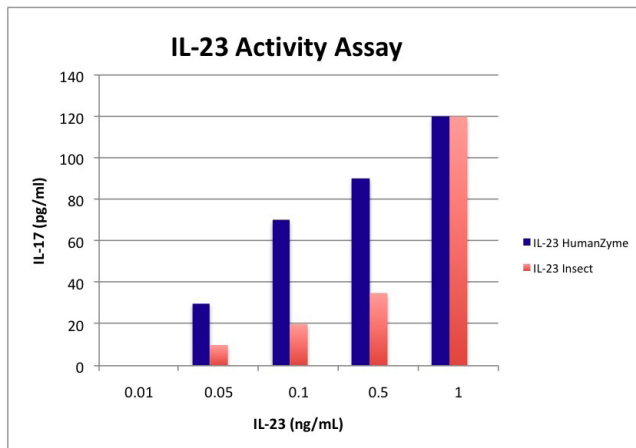


Fig. 2. IL-23 activities determined by the dose-dependent secretion of IL-17 from mouse splenocytes activated with 10 ng/ml PMA