

# Effective Polarization of Human Th17 Cells with Biologically Relevant HumaXpress™ TGFβ1, β2 and β3 Expressed in Human Cells



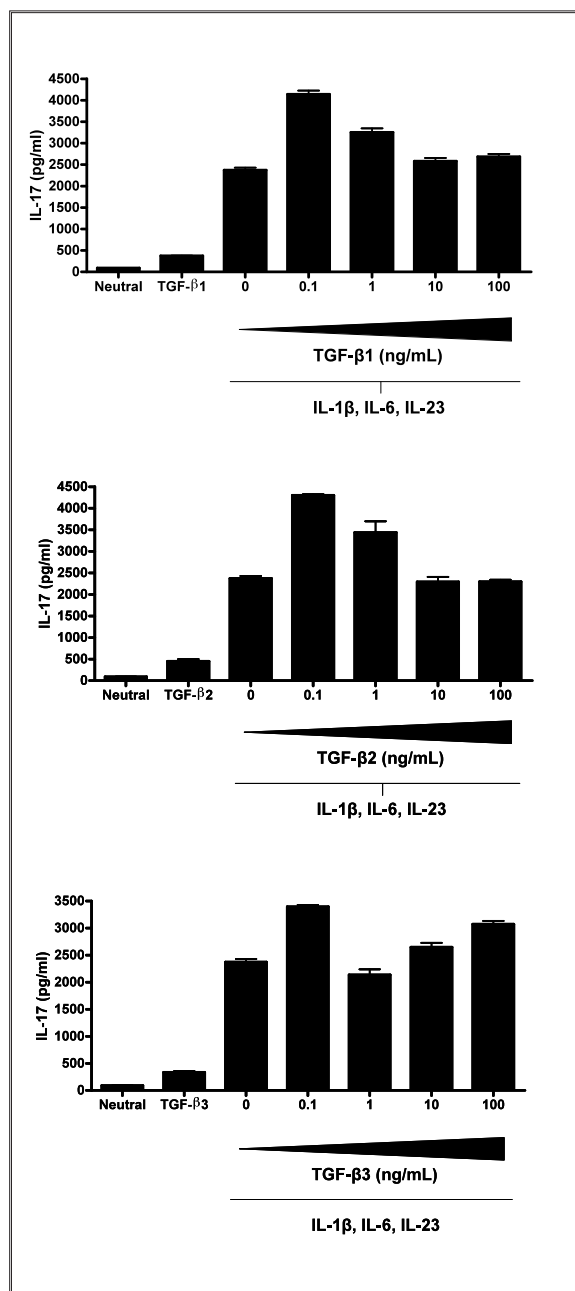
## INTRODUCTION

Transforming growth factors beta (TGF-β) are highly pleiotropic cytokines that act as cellular switches and regulate immune function, proliferation and epithelial-mesenchymal transition. These proteins are produced as precursors. A furin-like convertase processes the proprotein to generate an N-terminal latency-associated peptide (LAP) and a C-terminal mature TGF-β. Disulfide-linked homodimers of LAP and TGF-β remain non-covalently associated after secretion, forming the small latent TGF-β complex. Covalent linkage of LAP to latent TGF-β binding proteins create large latent complex that may interact with the extracellular matrix. Commercially available TGF-β proteins are produced as a recombinant protein expressed in CHO cells or as purified native protein from human platelets. Due to complex post-proteolytic modifications, TGF-β yield is low and the products are not available in economic bulk quantity. HumanZyme has developed an efficient human-cell based technology, HumaXpress™, for scalable production of human cytokines and produces TGF-β1, β2 and β3 (All HuXp™) from engineered human 293 cells. The proteins are highly purified disulfide-linked dimers of 25 kD that can be cost-effectively produced in large scale.

## TH17 POLARIZATION

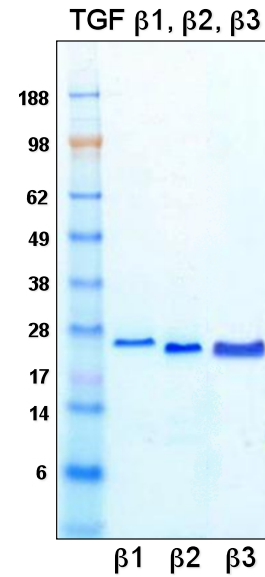
TGF-βs, which are important for the polarization of murine TH17 cells, are reported not required, and are even inhibitory, for human Th17 polarization<sup>1,2</sup>. In this study, whole CD4<sup>+</sup> cells isolated from a healthy donor were stimulated with 10 μg/mL plate bound anti-CD3 and 10 μg/mL soluble anti-CD28 in the presence of Th17 polarizing cytokines from HumanZyme and another commercial vendor. After 5 days, supernatants were harvested for measurement of IL-17 by ELISA. The results show that all the TGF-βsHuXp are effective at inducing IL-17 secretion with optimal concentration of 0.1 ng/mL TGF-β.

In contrast, TGF-β1 from insect cells showed only marginal or even inhibitory effects. The results indicate that using biologically relevant cytokines can more effectively induce Th17 cell polarization



and lead to a more accurate scientific understanding of the 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, TGF-β1<sup>HuXp</sup>, TGF-β2<sup>HuXp</sup>, TGF-β3<sup>HuXp</sup>, IL-1 beta<sup>HuXp</sup>, IL-6<sup>HuXp</sup>, and IL-23<sup>HuXp</sup>, are available in trial size and in bulk.



Purified TGF-β1<sup>HuXp</sup>, TGF-β2<sup>HuXp</sup>, and TGF-β3<sup>HuXp</sup> were resolved on an SDS-PAGE with Coomassie Blue staining.