

EPO^{HuXp} Exhibits Authentic Glycan Structure Expressed from Human 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. Cytokines generally alter the gene expression pattern of the target cell which leads 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 occur in the non-human cell expression systems. HumanZyme has developed an efficient human-cell based technology, HumaXpress[™] for scalable production of human cytokines.

EPO

Erythropoietin (EPO) is a 34 kD glycoprotein hormone which is related to thrombopoietin. This protein promotes erythrocyte formation by preventing the apoptosis of early erythroid precursors. It has been shown glycosylation of EPO is required for biological activities in vivo. Currently, commercially available recombinant human EPO proteins are produced from CHO cells. These recombinant proteins differ from

the native human EPO by having higher apparent molecular mass of 37 kD (as indicated by migration on SDS-PAGE) and lower content of neutral glycans. HumanZyme has produced EPO^{HuXp} from engineered human 293 cells. Similar to the native human protein, EPO^{HuXp} exhibits a lower apparent molecular mass and substantially higher content of neutral glycans. Furthermore, EPO^{HuXp} has more abundant and diverse glycan profiles than the CHO cell produced version. The most abundant glycans in EPO^{HuXp} are tetra-antennary complex types whereas those in CHO EPO are elongated bi-antennary complex types.

Comparison of Acidic Glycans of the recombinant EPO
from human vs CHO Cells

m/z [M-H] ⁻	Human	CHO
2441.87	 6.5%	 12.2%
2807.00	 28.4%	 58.5%
3172.13	 4.2%	 23.7%
2848.03	 38.0%	 3.4%

(See product catalog number HZ-1021, HZ-1022, and HZ-1023 at www.humanzyme.com)

HumanZyme has developed and continues to develop a growing group of tag-free cytokines, including difficult-to-express protein members of the TGFβ1 superfamily. HumanZyme Authentic Cytokines can be used as highly preferred reagents for cancer, inflammation, stem cell research, and antibody development.