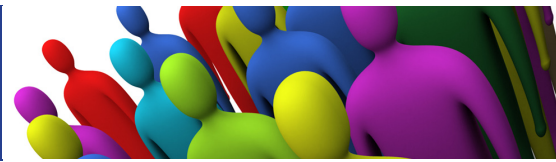


VEGF 165^{HuXp} Recombinant Human Cytokine Authentic - Human Cell Expressed



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 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*) 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. Currently, we have successfully produced expanding range of tag-free cytokines, including difficult-to-express protein members of the TGF β superfamily. As demonstrated below, HumanZyme's authentic cytokines can be used as highly preferred reagents for cancer, inflammation, stem cell research, and antibody development.

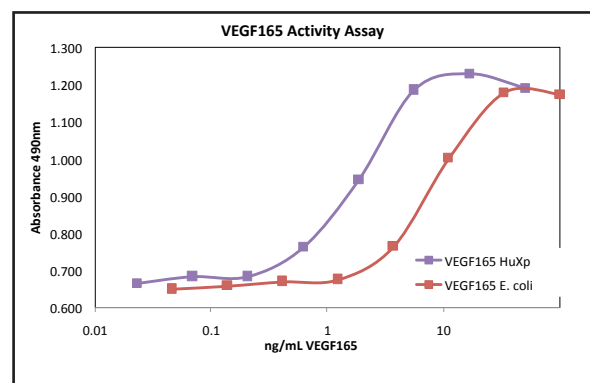
VEGF 165

VEGF165 is a member of the cysteine-knot growth factor superfamily. This cytokine stimulates proliferation and survival of endothelial cells, and promote angiogenesis and vascular permeability. Expressed in vascularized tissues, VEGF165 plays a prominent role in

normal and pathological angiogenesis. It has been demonstrated that inhibition of VEGF165 activity by treatment with a monoclonal antibody specific for VEGF165 can suppress tumor growth in vivo.

Currently, commercially available VEGF165 proteins are produced from non-human cells including *E. coli* and insect cells. HumanZyme has produced VEGF165^{HuXp} from engineered human 293 cells. The *E. coli* expressed protein is a monomer - dimer mixture and has a molecular mass of 18 and 34 kD in SDS-PAGE. This compares with the VEGF165^{HuXp} which migrates as a glycosylated band of 36 kD due to glycosylation and dimerization.

The bioactivity of VEGF165^{HuXp} was determined by its ability to induce proliferation of human umbilical vein endothelial cells. These results indicate VEGF165^{HuXp} is 5-fold more active than the *E. coli* expressed protein (ED_{50} of 1.6



ng/mL for HumaXpress[™] expressed protein vs 7.9 ng/mL for *E. coli* expression. (See product catalog number HZ-1013 and HZ-1038 at www.humanZyme.com)