

## RECOMBINANT HUMAN INTERLUEKIN 6 (IL-6)

Interleukin 6 (IL-6) is a soluble mediator with a pleiotropic effect on inflammation, immune response, and hematopoiesis. It is promptly and transiently produced in response to infections and tissue injuries, contributes to host defense through the stimulation of acute phase responses, hematopoiesis, and immune reactions. IL-6 produces inflammatory effects by inducing the transcription of factors in multiple pathways of inflammation. These may originate with protein kinase C, cAMP/ protein kinase A, and the release of calcium<sup>1,2</sup>.

IL-6 expression is observed in various cell types, such as monocytes, fibroblasts, endothelial cells, T cells, etc. IL6 in T-cells help in differentiation leading to its activation to achieve pathogen lysis inside the cells. IL-6 also helps B-cells differentiate, proliferate, and promote the formation of plasma cells from B-cells. In addition, as a growth factor for these cells, IL-6 enhances antibody release in the form of immunoglobulin A (IgA) and IgG. IL-6 is released by monocytes and macrophages in response to other inflammatory cytokines which include IL-11 and tumor necrosis factor (TNF)-beta. The IL-6 receptor is present on normal T-lymphocytes in the resting



phase, normally activated B-cells, and cells in the myeloid and hepatic cell lines. IL-6 is also found on B cells modified by the Epstein-Barr virus. It appears that IL-6 plays an important role in the development of Kaposi's sarcoma and multiple myeloma<sup>1,2</sup>.

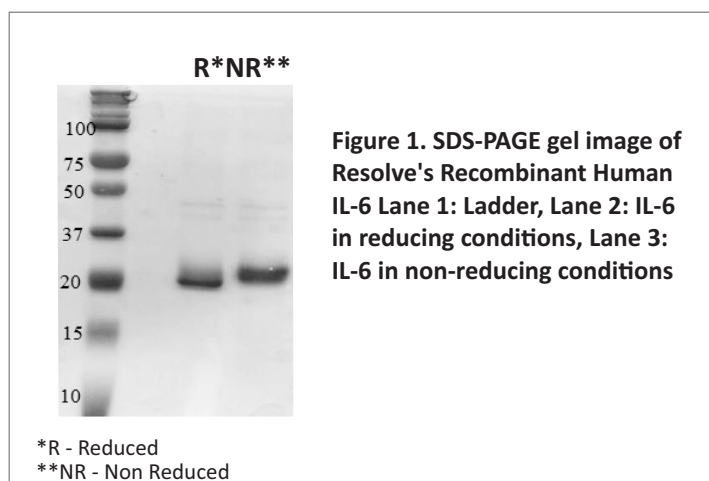
IL-6 has multiple diverse applications in culturing of primary cells, replacement of feeder cells in the preparation of murine and human hybridomas. Recombinant Human IL-6 from Resolve Biotech is a biologically active 24.3 kDa protein, expressed in E.coli and purified using conventional affinity chromatography technique.

## Technical Specifications

### A. Purity:

#### 1. SDS-PAGE

Resolve's Recombinant Human IL-6 is >95% pure as determined by reducing and non-reducing SDS-PAGE (Figure 1).



## B. Antigenic Activity – ELISA:

Antigenic activity for Resolve's Recombinant Human IL-6 was tested using Elabscience ELISA Kit (Cat No. E-EL-H6156). Serial dilutions of Resolve's Recombinant Human IL-6 (6.25–50 ng/mL) were assayed using the ELISA kit. The sample was found to have comparable activity/concentration as determined by extrapolating using the ELISA standard curve (Figure 2).

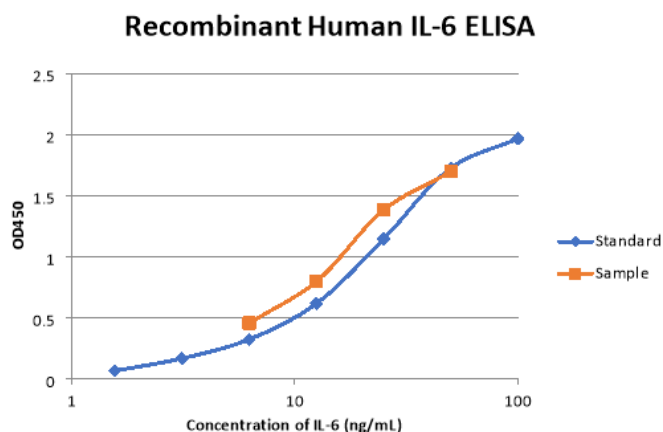


Figure 2: Result of IL-6 ELISA assay. Standard plot of IL-6 overlaid with Resolve's Recombinant Human IL-6 sample dilutions using IL-6 ELISA (Elabscience Catalog E-EL-H6156)

## C. Biological Activity:

IL-6 is capable of increasing proliferation of multiple myeloma cell line RPMI 8226<sup>3 4</sup> via autocrine and/or paracrine mechanisms. IL-6 mediates this function through a receptor composed of two membrane subunits: an  $\alpha$  chain (the IL-6 binding protein IL-6R) and a  $\beta$  chain (the signal transducer gp130). This complex leads to an activation of JAKs/STAT and RAS/MAPKs pathways. Janus kinases (JAKs) and the signal transducer-activator of transcription (STAT) regulate survival. RAS/mitogen-activated protein kinase (MAPK) is involved in the proliferation.

Resolve's Recombinant Human IL-6 is a biologically active protein as measured in a cell proliferation assay using RPMI 8226 cells. (Figure 3). The ED50 for this effect is <2 ng/mL.

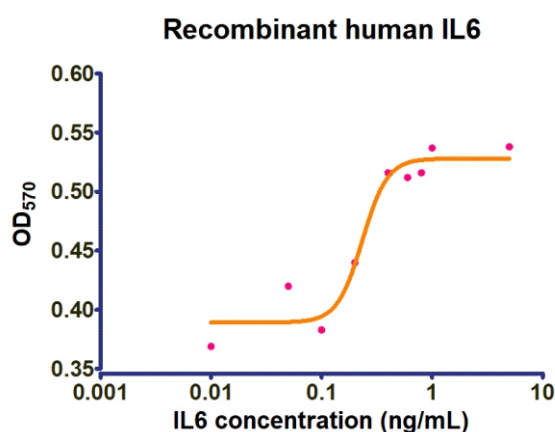


Figure 3. IL-6 mediated proliferation assay using RPMI 8226 cells

## References

<sup>1</sup> Tanaka, T. et. al. IL-6 in Inflammation, Immunity, and Disease. Cold Spring Harb Perspect Biol. 2014 Oct;6(10):a016295

<sup>2</sup> Tanaka, T. et. al. The Biology and Medical Implications of Interleukin-6. Cancer Immunol Res (2014) 2 (4): 288–294.

<sup>3</sup> Barut, B.B. et. al., Role of Interleukin 6 in the growth of myeloma derived cell lines. Leukemia Research, 1992 Vol 16(10), pp 951-959

<sup>4</sup> Kovacs, Eva. Multiple Myeloma and B Cell Lymphoma Investigation of IL-6, IL-6 Receptor Antagonist(IL-6RA), and GP130 Antagonist (GP130A) Using Various Parameters in an In Vitro Model. The Scientific World Journal, 2006 Vol 6, pp 888-898

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