

# Recombinant Human Interleukin-12 (rHuIL-12)

## Acnovia Data Sheet

<b>Catalog#/Size:</b>	AC52382/100 µg.
<b>Source:</b>	CHO-driven Recombinant Human Interleukin-12.
<b>Molecular Weight:</b>	Approximately 59 kDa (p40-Linker-p35) , containing 523 amino acids.
<b>Description :</b>	Accession # P29460 Human IL-12 p40 (Ile23-Ser328)&Accession # P29459 Human IL-12 p35 (Arg23-Ser219) with a N terminal Ile.
<b>SDS-PAGE:</b>	72 kDa, under reducing and no reducing condition.
<b>Purity:</b>	> 95 % , as determined by SDS-PAGE, under reducing non-reducing conditions, visualized by coomassie staining.
<b>Endotoxin:</b>	Less than 0.01 EU/µg of rHuIL-12 as determined by kinetic Limulus Amoebocyte Lysate (LAL) assay.
<b>Biological Activity:</b>	Recombinant human IL-12 bioactivity is measured in a cell proliferation assay using PHA-P-activated human peripheral blood mononuclear cell (PBMC), the EC50 for this effect is 0.003197-0.006955 ng/mL.
<b>Physical Appearance:</b>	Sterile Filtered White lyophilized (freeze-dried) powder.
<b>Formulation:</b>	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose.
<b>Reconstitution:</b>	We recommend that this vial be briefly centrifuged prior to opening to bring the contents to the bottom. Reconstitute to a concentration of 0.1-1.0 mg/mL in sterile distilled H <sub>2</sub> O. Stock solutions should be apportioned into working aliquots and stored at -20 °C to -70 °C. Further dilutions should be made in appropriate buffered solutions. Do not reconstitute in cell culture media directly.
<b>Shipping:</b>	The product is shipped at 2 °C to 8 ° C. Upon receipt, store it immediately at the temperature recommended below.
<b>Stability &amp; Storage:</b>	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. A minimum of 12 months from date of shipping when stored at -20 °C to -70 °C as supplied. Refer to lot specific COA for the use by date. 4 weeks at 2 °C to 8 °C under sterile conditions after reconstitution. 4 months at -20 °C to -70 °C under sterile conditions after reconstitution
<b>Usage:</b>	Acnovia rHuIL-12 product can be used for a variety of ex vivo cell culture applications such as research or further manufacturing.
<b>Quality statement:</b>	No animal- or human-derived materials were used for the manufacture of this product, unless otherwise stated in the respective Certificate of Origin.

### Background:

Interleukin-12 (IL-12) is a heterodimeric cytokine of 70 kDa (p70 protein) comprising two covalently linked subunits p35 (35 kDa) alpha (α) chain and the p40 (40 kDa) beta (β) chain. The p40 chain is overproduced relative to the p35 chain and forms homodimers that bind to the IL-12 receptor, competing with the bioactive p70 heterodimer for receptor occupancy and thereby serving as a receptor antagonist. IL-12 is predominantly produced by activated dendritic cells (DCs), macrophages, and neutrophils during T-cell priming and acts directly on cytotoxic immune effector cells, including natural killer (NK) cells, natural killer T (NKT) cells, and CD8+ T cells, to stimulate their proliferation and increase their cytotoxic functions. IL-12 is widely accepted as an important regulator of Th1 responses. It also promotes the expansion and survival of activated T-cells and NK cells and modulates the cytotoxic activity of CTLs and NK cells.

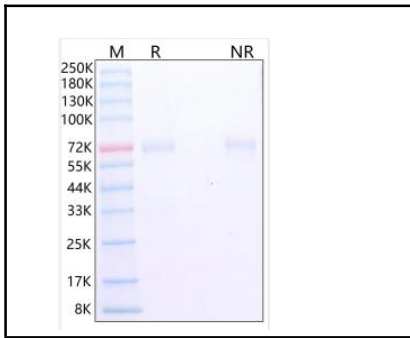
During the adaptive immune response, IL-12 primes antigen-specific T-cells for high IFN-γ production (driving their differentiation toward the Th-1 pathway). IL-12 can also act as an adjuvant for humoral immunity by enhancing production of IgG2a and IgG2b antibodies.

Recombinant human IL-12 is a 69-75 kDa isodimer glycoprotein (total of 503 amino acid residues) composed of 35 kDa (p35) and 40 kDa (p40) subunits linked by disulfide bonds.

### Application References:

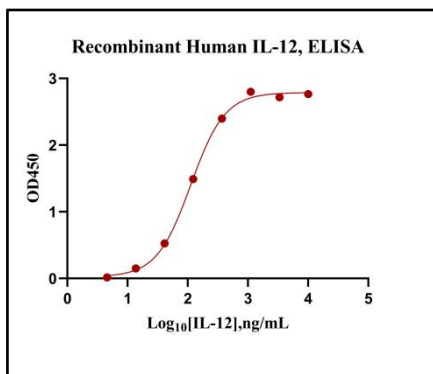
1. Violeta Rus, Charles S. Via, Chapter 12 - Cytokines in Systemic Lupus Erythematosus, Editor(s): George C. Tsokos, Caroline Gordon, Josef S. Smolen, Systemic Lupus Erythematosus, Mosby, 2007, Pages 109-120;
2. Greiner JW, Morillon YM 2nd, Schlom J. NHS-IL12, a Tumor-Targeting Immunocytokine. *Immunotargets Ther.* 2021 May 27;10:155-169.

## DATA:



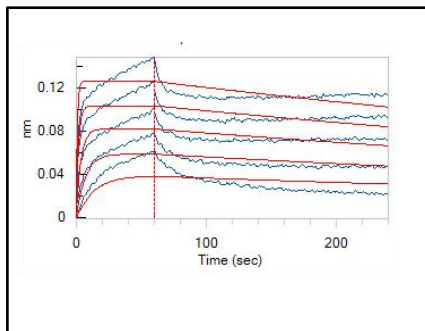
### SDS- PAGE

1  $\mu$ g/lane of Recombinant Human IL-12 Protein (Catalog #AC52382) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions visualized by coomassie staining showing a single band at 72 kDa under non-reducing conditions and reducing condition.



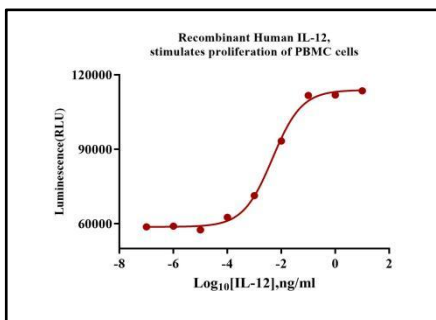
### Bioactivity-ELISA

Immobilized Recombinant human IL-12 (Catalog #AC52382) at 0.2  $\mu$ g/well can bind human IL-12R with a linear range of 97.89 to 128.2 ng/mL.



### Bioactivity-BLI

Loaded Human IL-12R can bind Recombinant Human IL-12 (Catalog #AC52382) with an affinity constant of 0.98 nM as determined in BLI assay (Octet®R8).



### Bioactivity- Cell based assay

Recombinant Human IL-12 (Catalog #AC52382) stimulates proliferation of PHA-P-activated human peripheral blood mononuclear cell (PBMC). The EC<sub>50</sub> for this effect is 0.003197 to 0.006955 ng/mL.