

**DESCRIPTION**

<b>Source</b>	<i>E. coli</i> -derived human IL-4 protein His25-Ser153, with an N-terminal Met Accession # P05112 Produced using non-animal reagents in an animal-free laboratory. Manufactured and tested under cGMP guidelines.
<b>N-terminal Sequence Analysis</b>	Met-His <sub>25</sub> -Lys-(Cys)-Asp-Ile-Thr-Leu-Gln-Glu
<b>Predicted Molecular Mass</b>	15.1 kDa

**SPECIFICATIONS**

<b>SDS-PAGE</b>	14 kDa, reducing conditions
<b>Activity</b>	Measured in a cell proliferation assay using TF-1 human erythroleukemic cells. Kitamura, T. <i>et al.</i> (1989) <i>J. Cell Physiol.</i> <b>140</b> :323. The ED <sub>50</sub> for this effect is 0.05-0.2 ng/mL.  The specific activity of recombinant human IL-4 is >1.0 x 10 <sup>7</sup> IU/mg, which is calibrated against the human IL-4 WHO International Standard (NIBSC code: 88/656).
<b>Endotoxin Level</b>	<0.10 EU per 1 µg of the protein by the LAL method.
<b>Purity</b>	>97%, by SDS-PAGE under reducing conditions and visualized by silver stain.
<b>Host Cell Protein</b>	< 0.5 ng per µg of protein when tested by ELISA.
<b>Mycoplasma</b>	Negative when tested in a ribosomal RNA hybridization assay.
<b>Formulation</b>	Lyophilized from a 0.2 µm filtered solution in PBS. See Certificate of Analysis for details.

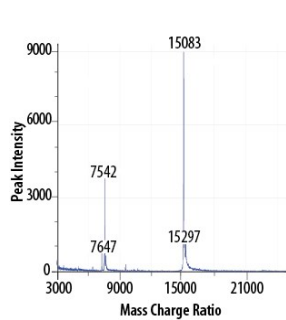
**PREPARATION AND STORAGE**

<b>Reconstitution</b>	Reconstitute at 100-200 µg/mL in PBS.
<b>Shipping</b>	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>A minimum of 12 months when stored at ≤ -20 °C as supplied. Refer to lot specific COA for the Use by Date.</li> <li>1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>3 months, ≤ -20 °C under sterile conditions after reconstitution.</li> </ul>

**DATA**

<p><b>Bioactivity</b></p> <p>GMP-grade Recombinant Human IL-4 (Catalog # 204-GMP) stimulates proliferation of TF-1 human erythroleukemic cells. The ED<sub>50</sub> is 0.05-0.2 ng/mL.</p>	<p><b>SDS-PAGE</b></p> <p>1 µg/lane of GMP-grade Recombinant Human IL-4 (Catalog # 204-GMP) was resolved with SDS-PAGE under reducing (R) conditions and visualized by silver staining, showing a single band at 14 kDa.</p>
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**Mass Spectrometry**



MALDI-TOF analysis of GMP-grade Recombinant Human IL-4 (Catalog # 204-GMP). The major peak at 15083 Da corresponds to the calculated molecular mass, 15094 Da. The minor peak at 15297 Da is a matrix-associated artifact of the MALDI-TOF.

**BACKGROUND**

Interleukin-4 (IL-4), also known as B cell-stimulatory factor-1, is a monomeric, approximately 13 kDa-18 kDa Th2 cytokine that shows pleiotropic effects during immune responses (1-3). It is a glycosylated polypeptide that contains three intrachain disulfide bridges and adopts a bundled four  $\alpha$ -helix structure (4). Human IL-4 is synthesized with a 24 aa signal sequence. Alternate splicing generates an isoform with a 16 aa internal deletion. Mature human IL-4 shares 55%, 39% and 43% aa sequence identity with bovine, mouse, and rat IL-4, respectively. Human, mouse, and rat IL-4 are species-specific in their activities (5-7). IL-4 exerts its effects through two receptor complexes (8, 9). The type I receptor, which is expressed on hematopoietic cells, is a heterodimer of the ligand binding IL-4 Ra and the common  $\gamma$  chain (a shared subunit of the receptors for IL-2, -7, -9, -15, and -21). The type II receptor on nonhematopoietic cells consists of IL-4 Ra and IL-13 Ra1. The type II receptor also transduces IL-13 mediated signals. IL-4 is primarily expressed by Th2-biased CD4<sup>+</sup> T cells, mast cells, basophils, and eosinophils (1, 2). It promotes cell proliferation, survival, and immunoglobulin class switch to IgG4 and IgE in human B cells, acquisition of the Th2 phenotype by naïve CD4<sup>+</sup> T cells, priming and chemotaxis of mast cells, eosinophils, and basophils, and the proliferation and activation of epithelial cells (10-13). IL-4 plays a dominant role in the development of allergic inflammation and asthma (12, 14).

**References:**

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**MANUFACTURING SPECIFICATIONS**

**GMP Proteins**

R&D Systems, a Bio-Techne Brand's GMP proteins are produced according to relevant sections of the following documents: WHO TRS, No. 822, 1992 Annex 1, Good Manufacturing Practices for Biological Products; USP Chapter 1043, Ancillary Materials for Cell, Gene and Tissue-Engineered Products and USP Chapter 92, Growth Factors and Cytokines Used in Cell Therapy Manufacturing.

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- Equipment calibration schedules using a computerized calibration program
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- Material review process for variances
- Monitoring of stability over product shelf-life

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- N-terminal amino acid analysis, SDS-PAGE analysis, and endotoxin level (as determined by LAL assay) performed on each bulk QC lot, not on individual bottlings of each QC lot
- Post-bottling lot-specific bioassay results (compliance with an established range) and results of microbial testing according to USP
- Host Cell Protein testing performed by ELISA
- Mycoplasma testing by ribosomal RNA hybridization assay

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