

#### DESCRIPTION

**Source** *E. coli*-derived human IL-15 protein  
Asn49-Ser162  
Accession # P40933.1  
Produced using non-animal reagents in an animal-free laboratory.  
Manufactured and tested under cGMP guidelines.

**N-terminal Sequence Analysis** Asn49-Trp-Val-Asn-Val-Ile-Ser-Asp-Leu-Lys

**Predicted Molecular Mass** 13 kDa

#### SPECIFICATIONS

**SDS-PAGE** 9 kDa, under reducing conditions

**Activity** Measured in a cell proliferation assay using MO7e human megakaryocytic leukemic cells.  
The ED<sub>50</sub> for this effect is 0.300-2.60 ng/mL. The specific activity of recombinant human IL-15 is >2.00 x 10<sup>8</sup> units/mg, which is calibrated against the human IL-15 reference standard (NIBSC code: 95/554).

**Endotoxin Level** <0.10 EU per 1 µg of the protein by the LAL method.

**Purity** >97%, by SDS-PAGE with quantitative densitometry by Coomassie® Blue Staining.

**Mass Spectrometry** The molecular weight by mass spectrometry is 12761 Da ± 5 Da.

**Host Cell Protein** <1.00 ng per µg of protein when tested by ELISA.

**Mycoplasma** Negative for Mycoplasma.

**Host Cell DNA** <0.0100 ng per µg of protein when tested by PCR.

**Formulation** Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.

#### PREPARATION AND STORAGE

**Reconstitution** Reconstitute at 100-500 µg/mL in PBS.

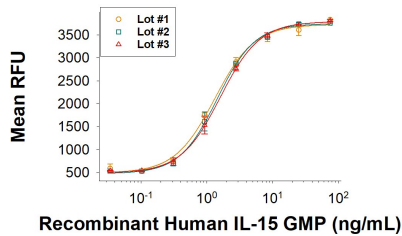
**Shipping** The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

**Stability & Storage** **Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**

- A minimum of 12 months when stored at ≤ -20 °C as supplied. Refer to lot specific COA for the Use by Date.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 3 months, ≤ -20 °C under sterile conditions after reconstitution.

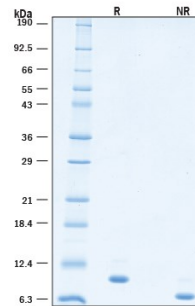
**DATA**

**Bioactivity**



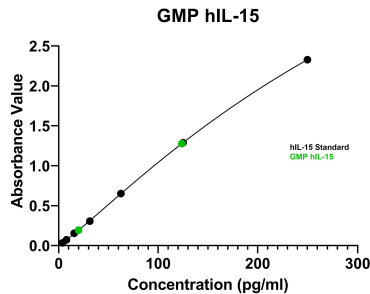
**Recombinant Human IL-15 Protein GMP Bioactivity.** GMP-grade Recombinant Human IL-15 (Catalog # BT-015-GMP) stimulates cell proliferation in the MO7e human megakaryocytic leukemic cell line. The ED<sub>50</sub> for this effect is 0.300-2.60 ng/mL. Three independent lots were tested for activity and plotted on the same graph to show lot-to-lot consistency of GMP IL-15.

**SDS-PAGE**



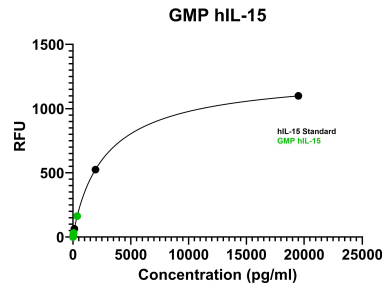
**Recombinant Human IL-15 GMP Protein SDS-PAGE 2** µg/lane of GMP-grade Recombinant Human IL-15 (Catalog # BT-015-GMP) was resolved with SDS-PAGE under reducing (R) conditions and visualized by Coomassie® Blue staining, showing a single band at 9 kDa.

**ELISA**



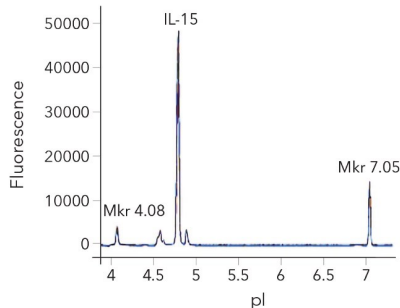
**Recombinant Human IL-15 GMP Protein Measured by Quantikine ELISA.** Two samples of GMP-grade human IL-15 (Catalog number BT-015-GMP, green circles) were interpolated with the human IL-15 Quantikine ELISA (Catalog # D1500) standard curve (black circles) using 4PL regression analysis. The human IL-15 Quantikine ELISA has an assay range of 3.9-250 pg/mL.

**ELISA**



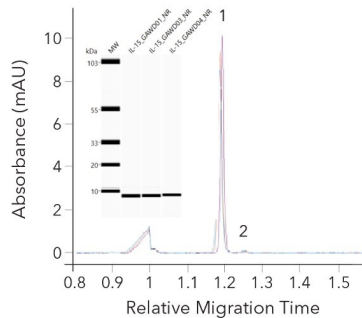
**Recombinant Human IL-15 GMP Protein Measured by ELLA.** Thirteen samples of GMP-grade human IL-15 (Catalog number BT-015-GMP, green circles) were interpolated with the Simple Plex Human IL-15 cartridge (Catalog # SPCKB-PS-000500) standard curve (black circles) using 4PL logistic regression analysis. The Simple Plex IL-15 cartridge has an assay range of 0.2-1,950 pg/mL.

**Data**



**Recombinant Human IL-15 GMP Protein Purity Analysis by Charge Variation.** Three independent lots of Recombinant Human IL-15 GMP Protein (Catalog # BT-015-GMP) were analyzed by Maurice icIEF using native fluorescence detection (Mkr 5.85 and 9.99 are pI Markers). Profiles from the three runs were superimposed, showing excellent manufacturing consistency.

**Data**



**Recombinant Human IL-15 GMP Protein Purity Analysis by Size Separation.** Three independent lots of Recombinant Human IL-15 GMP Protein (Catalog # BT-015-GMP) were analyzed by Maurice CE-SDS PLUS (IS is an Internal Standard). A gel-like representation of the purity analysis data (inset) can be obtained from the Lane View feature in Compass software for iCE. Profiles from the three runs were superimposed, showing excellent manufacturing consistency.

## BACKGROUND

Interleukin 15 (IL-15) is a widely expressed 14 kDa cytokine that is structurally and functionally related to IL-2 and plays an important role in many immunological diseases (1, 2). Mature human IL-15 shares 70% amino acid sequence identity with mouse and rat IL-15. Alternative splicing generates isoforms of IL-15 with either a long or short signal peptide (LSP or SSP), and the SSP isoform is retained intracellularly (3). IL-15 binds with high affinity to IL-15 R $\alpha$  (4). It binds with lower affinity to a complex of IL-2 R $\beta$  and the common gamma chain ( $\gamma$ c) which are also subunits of the IL-2 receptor complex (5). IL-15 associates with IL-15 R $\alpha$  in the endoplasmic reticulum, and this complex is expressed on the cell surface (6).

The dominant mechanism of IL-15 action is known as transpresentation in which IL-15 and IL-15 R $\alpha$  are coordinately expressed on the surface of one cell and interact with complexes of IL-2 R $\beta$ / $\gamma$ c on adjacent cells (7). This enables cells to respond to IL-15 even if they do not express IL-15 R $\alpha$  (6). In human and mouse, soluble IL-15-binding forms of IL-15 R $\alpha$  can be generated by proteolytic shedding and bind up nearly all the IL-15 in circulation (8-10). Soluble IL-15 R $\alpha$  functions as an inhibitor that limits IL-15 action (4, 9). Ligation of membrane-associated IL-15/IL-15 R $\alpha$  complexes also induces reverse signaling that promotes activation of the IL-15/IL-15 R $\alpha$  expressing cells (11). IL-15 induces or enhances the differentiation, maintenance, or activation of multiple T cell subsets including NK, NKT, Th17, Treg, and CD8+ memory cells (12 - 16). An important component of these functions is the ability of IL-15 to induce dendritic cell differentiation and inflammatory activation (11, 14). IL-15 exhibits anti-tumor activity independent of its actions on NK cells or CD8+ T cells (17). It also inhibits the deposition of lipid in adipocytes, and its circulating levels are decreased in obesity (18).

Immunotherapy treatment with recombinant IL-15 has the advantage of not stimulating Treg cells like IL-2 does but has the drawback of associated toxicity at higher doses. This has led to increased investigation on mitigating IL-15 toxicity and combination immunotherapy approaches using immune checkpoint inhibitors (19, 20). Preclinical and early clinical studies have shown the potential of also using IL-15 in combination with cancer vaccines to improve their anti-tumor response (20).

IL-15 can also be used for the preconditioning of CAR T cells or for engineering cells to express IL-15 in vivo. Adoptive cell transfer of NK cells engineered to express CD19 and IL-15 were well tolerated in patients with CD19-positive cancers (20). IL-15 can be used in combination with other cytokines like IL-21 to increase the efficiency of NK cell expansion and maturation in stem cell culture protocols (21). The combination of IL-15 with IL-7 also promotes expansion of early-differentiated CD8+ T cells in culture with the added benefit of decreasing Treg cell generation, unlike IL-2, for adoptive cell transfer in cancer immunotherapy (22). GMP IL-7 and GMP IL-15 are commonly used in combination for ex vivo expansion of T cells for cellular therapies.

## References:

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## MANUFACTURING SPECIFICATIONS

R&D Systems, a Bio-Techne Brand's GMP proteins are produced according to relevant sections of the following documents: USP Chapter 1043, Ancillary Materials for Cell, Gene and Tissue-Engineered Products and Eu. Ph. 5.2.12, Raw Materials of Biological Origin for the Production of Cell-based and Gene Therapy Medicinal Products.

R&D Systems' quality focus includes:

- Manufactured and tested under an ISO 9001:2015 and ISO 13485:2016 certified quality system
- Documented processes and QA control of documentation and process changes
- Personnel training programs
- Raw material testing and vendor qualification/monitoring
- Fully validated equipment, processes and test methods
- Equipment calibration schedules using a computerized calibration program
- Facility maintenance, safety programs and pest control
- Material review process for variances
- Monitoring of stability over product shelf-life

R&D Systems strives to provide our customers with the analytical characteristics of each product so that customers may determine whether our products are appropriate for their research. The Certificate of Analysis provided contains the following lot specific information:

- 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 <71>
- Host Cell Protein testing performed by ELISA
- Mycoplasma testing by ribosomal RNA hybridization assay

Additional testing and documentation requested by the customer can be arranged at an additional cost.

Production records and facilities are available for examination by appropriate personnel on-site at R&D Systems.

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### Production

- All molecular biology procedures use animal-free media and dedicated labware.
- Dedicated fermentors are utilized in committed animal-free areas.

### Purification

- Protein purification columns are animal-free.
- Bulk proteins are filtered using animal-free filters.
- Purified proteins are stored in animal-free containers in a dedicated cold storage room.

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- Low Endotoxin Level.
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