

DESCRIPTION

Source	<i>E. coli</i> -derived human IL-2 protein Ala21 - Thr153 (Cys145Ser), with and without an N-terminal Met Accession # P60568.1 Produced using non-animal reagents in an animal-free laboratory. Manufactured and tested under cGMP guidelines.
N-terminal Sequence Analysis	Met-Ala21-Pro-Thr-Ser-Ser-Ser-Thr-Lys-Lys & Ala21-Pro-Thr-Ser-Ser-Ser-Thr-Lys-Lys-Thr
Predicted Molecular Mass	15.5 kDa

SPECIFICATIONS

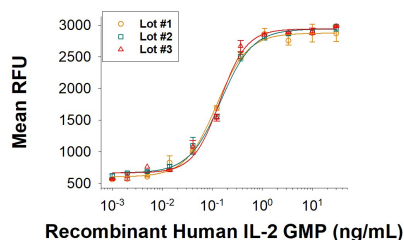
SDS-PAGE	13 kDa, under reducing conditions.
Activity	Measured in a cell proliferation assay using CTLL-2 mouse cytotoxic T cells. Gearing, A.J.H. and C.B. Bird (1987) in <i>Lymphokines and Interferons, A Practical Approach</i> . Clemens, M.J. <i>et al.</i> (eds): IRL Press. 295. The ED ₅₀ for this effect is 0.0300-0.250 ng/mL. The specific activity of recombinant human IL-2 is >5.00 x 10 ⁶ IU/mg, which is calibrated against an internal reference standard value assigned against the human IL-2 WHO International Standard (NIBSC code: 86/500).
Endotoxin Level	<0.01 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 15521 ± 5 Da, and a second 15390 ± 5 Da product may be present.
Host Cell Protein	<0.500 ng per µg of protein when tested by ELISA.
Mycoplasma	Negative for Mycoplasma.
Host Cell DNA	<0.00150 ng per µg of protein when tested by PCR.
Formulation	Lyophilized from a 0.2 µm filtered solution in Sodium Acetate with Trehalose. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 500 µg/mL in sterile deionized water.
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. <ul style="list-style-type: none"> • 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.

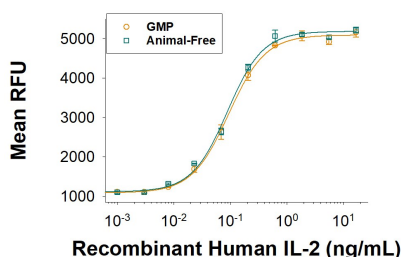
DATA

Bioactivity



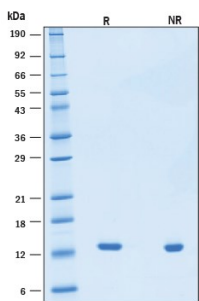
Recombinant Human IL-2 GMP Protein Bioactivity. GMP-grade Recombinant Human IL-2 (Catalog # BT-002-GMP) as measured in a cell proliferation assay using CTLL-2 mouse cytotoxic T cells. The ED₅₀ for this effect is 0.0300-0.250 ng/mL. Three independent lots were tested for activity and plotted on the same graph to show lot-to-lot consistency of GMP IL-2.

Bioactivity



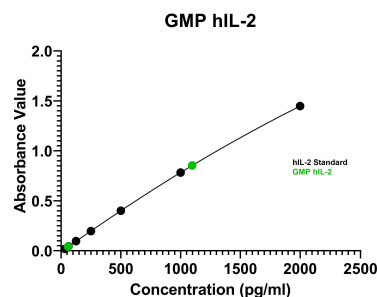
Equivalent Bioactivity of GMP and Animal-Free grades of Recombinant Human IL-2. Equivalent bioactivity of GMP (Catalog # BT-002-GMP) and Animal-Free (Catalog # BT-002-AFL) grades of Recombinant Human IL-2 as measured in a cell proliferation assay using CTLL-2 mouse cytotoxic T cells (orange and green, respectively).

SDS-PAGE



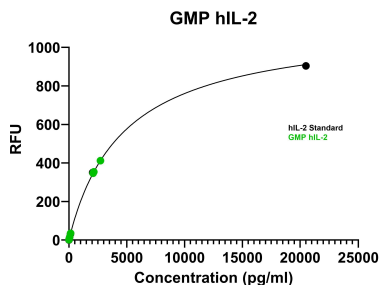
Recombinant Human IL-2 GMP Protein SDS-PAGE. 2 µg/lane of Recombinant Human IL-2 GMP Protein (Catalog # BT-002-GMP) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 13 kDa.

ELISA



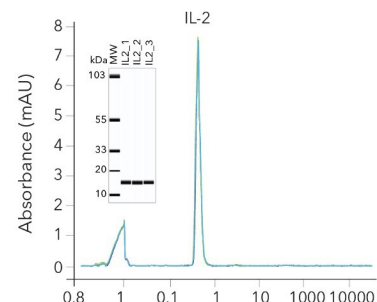
Recombinant Human IL-2 GMP Protein Measured by Quantikine ELISA. Two samples of GMP-grade human IL-2 (Catalog number BT-002-GMP, green circles) were interpolated with the human IL-2 Quantikine ELISA (Catalog # D2050) standard curve (black circles) using 4PL logistic regression analysis. The human IL-2 Quantikine ELISA has an assay range of 31.2-2,000 pg/mL.

ELISA



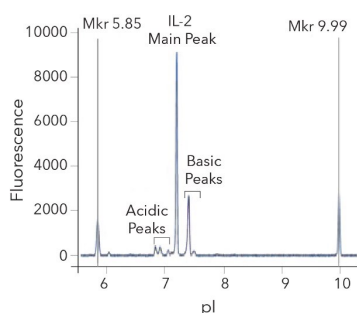
Recombinant Human IL-2 GMP Protein Measured by ELISA. Thirteen samples of GMP-grade human IL-2 (Catalog number BT-002-GMP, green circles) were interpolated with the Simple Plex Human IL-2 cartridge (Catalog # SPCKB-PS-000295) standard curve (black circles) using 4PL logistic regression analysis. The Simple Plex IL-2 cartridge has an assay range of 0.54-2,050 pg/mL.

Data



Recombinant Human IL-2 GMP Protein Purity Analysis by Size Separation. Three independent lots of Recombinant Human IL-2 GMP Protein (Catalog # BT-002-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.

Data



Recombinant Human IL-2 GMP Protein Purity Analysis by Charge Variation. Three independent lots of Recombinant Human IL-2 GMP Protein (Catalog # BT-002-GMP) were analyzed by Maurice iCE using native fluorescence detection (Mkr 5.85 and 9.99 are pI Markers). Profiles from the three runs were superimposed, showing excellent manufacturing consistency.

BACKGROUND

Recombinant Interleukin-2 (IL-2) is expressed in *E. coli* and has been engineered to contain the serine for cysteine substitution found in Proleukin® (aldesleukin). Recombinant IL-2 is widely used in cell culture for the expansion of T cells.

IL-2 is expressed by CD4⁺ and CD8⁺ T cells, $\gamma\delta$ T cells, B cells, dendritic cells, and eosinophils (1 - 3). Mature human IL-2 shares 56% and 66% amino acid (aa) sequence identity with mouse and rat IL-2, respectively. Human and mouse IL-2 exhibit cross-species activity (4). The receptor for IL-2 consists of three subunits that are present on the cell surface in varying preformed complexes (5 - 7). The 55 kDa IL-2 R α is specific for IL-2 and binds with low affinity. The 75 kDa IL-2 R β , which is also a component of the IL-15 receptor, binds IL-2 with intermediate affinity. The 64 kDa common gamma chain γ c/IL-2 R γ , which is shared with the receptors for IL-4, -7, -9, -15, and -21, does not independently interact with IL-2. Upon ligand binding, signal transduction is performed by both IL-2 R β and γ c.

IL-2 is best known for its autocrine and paracrine activity on T cells. It drives resting T cells to proliferate and induces IL-2 and IL-2 R α synthesis (1, 2). It contributes to T cell homeostasis by promoting the Fas-induced death of naïve CD4⁺ T cells but not activated CD4⁺ memory lymphocytes (8). IL-2 plays a central role in the expansion and maintenance of regulatory T cells, although it inhibits the development of Th17 polarized cells (9 - 11). Thus, IL-2 may be a key cytokine in the natural suppression of autoimmunity (12, 13).

IL-2 expression and concentration can have either immunostimulatory effects at high doses or immunosuppressive effects at low doses due to its preferential binding to different receptor subunits expressed by various immune cell types. This has led to the generation of recombinant IL-2 variants aimed at modifying IL-2 receptor binding for increased antitumor efficacy (14, 15). These variants are typically used in combination with immune checkpoint inhibitors instead of as a monotherapy (14). IL-2 can be genetically engineered to express in NK cells for CAR T cell therapies, and in combination with other cytokines like IL-15, can increase cell viability and proliferation (16). In addition to adoptive cell transfer and checkpoint blockade inhibitors, cancer vaccines that boost immune responses have been combined with IL-2 treatment with promising results in recent studies (15).

In cell culture, IL-2 is a frequently used cytokine for the proliferation, differentiation, and increased antibody secretion of B cells as they transform into plasma cells *in vitro* (17). IL-2 is also a classically used cytokine for the expansion of NK cells, early differentiated T cells and effector memory Treg cells for adoptive cell transfer cancer immunotherapy (16, 18). GMP IL-2 is a commonly used supplement for the expansion of these cell types for cellular therapies.

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: 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.

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- 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

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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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- Purified proteins are stored in animal-free containers in a dedicated cold storage room.

Quality Assurance

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