Interleukin-2, human (hIL-2)

recombinant *(E. coli)*

Solution, filtered through 0.2 μm pore size membrane

Version 17
Content version: February 2019
Store at −15 to −25°C

Cat. No. 10 799 068 001 10,000 U (5 μg, 50 ml)
Cat. No. 11 011 456 001 10,000 U (5 μg, 1 ml)
Cat. No. 11 147 528 001 50,000 U (25 μg, 5 ml)

1. What this Product Does

Contents

Cat. No.	U	μg	ml	U/ml	μg/ml
10 799 068 001	10,000	5	50	200	0.1
11 011 456 001	10,000	5	1	10,000	5
11 147 528 001	50,000	25	5	10,000	5

Solution in PBS (phosphate buffered saline) and 1 mg/ml BSA (bovine serum albumin), [purity of BSA: >98%, endotoxin (LAL): <1 EU/mg BSA], filtered through 0.2 μm pore size membrane.

Storage and Stability

Stable at −15 to −25°C until the expiration date printed on the label.
Store the solution in aliquots at −15 to −25°C.
Avoid repeated freezing and thawing.

Application

Recombinant IL-2, human allows

- the cultivation of human and murine IL-2 dependent T-cell lines and natural killer cell lines,
- the proliferation of mitogen-activated T-lymphocytes and natural killer cells,
- the establishment of human and murine thymocyte, splenocyte or peripheral blood lymphocyte (PBL) derived T-cell lines, and
- the generation of human and murine lymphokine-activated killer (LAK) cells (1-11).

2. How to Use this Product

2.1 Before you Begin

Working Concentration

Established IL-2-dependant T-cell lines usually require 10-20 U/ml. Add IL-2 to the freezing medium for IL-2 dependant cell lines.

Recommended Method of Dilution

Dilute the concentrated IL-2 solution (200 U/ml or 10,000 U/ml) with PBS or culture medium containing 1 mg/ml BSA or HSA (0.1%) or 1 – 10% serum.

Reagents Required

- Culture medium, *e.g.* RPMI 1640, containing 10% FCS, 10 mM Hepes*, 2 mM L-glutamine, (1×) non-essential amino acids, 1 mM sodium pyruvate and 50 mM β-mercaptoethanol.
- Cell Proliferation Kit II (XTT)*

2.2 Procedure

Instructions for the Determination of hIL-2 Activity on IL-2 Dependent Cells (IL-2 Proliferation Assay; Exemplary with CTLL-2 cells)

❶ Prepare hIL-2 test samples as serial dilutions (final concentration *e.g.,* from 0.001 – 100 ng/ml) in culture medium in a 96-well flat-bottomed microplate (tissue culture grade) in a volume of 100 μl.
As a negative control use culture medium alone.

❷ Harvest sensitive cells, *e.g.,* CTLL-2 cells (ATCC TIB 214) and wash them three times by centrifugation in culture medium without hIL-2.
Resuspend and adjust the cells in culture medium to 4×10^4 cells/ml.

❸ Add 100 μl of the cell suspension to 100 μl of the prediluted hIL-2 samples into each well, revealing a final cell number of 2×10^4 cells/ml (4×10^3 cells/well).

❹ Incubate the microplate for 48 h at 37°C and 5% CO_2.

❺ After the incubation period add 100 ml XTT labeling mixture [for details see working instruction of the Cell proliferation kit II (XTT)] to each well.
Incubate the microplate for another 6 h at 37°C and 5% CO_2.

❻ Measure the spectrophotometrical absorbance of the samples by using a microplate (ELISA) reader. To measure absorbance of the formazan product a wavelength between 450 and 500 nm (*e.g.,* 492 nm) can be used, depending on the optical filters available for the ELISA reader used.
The reference wavelength should be more than 650 nm.

3. Results

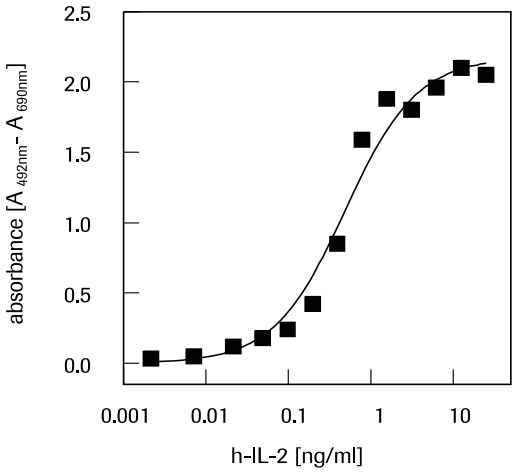

Fig. 1: Stimulation of cell proliferation with mouse CTLL-2 cells in response to recombinant IL-2, human (h-IL-2) using the procedure described.

4. Additional Information on this Product

Product Description

Interleukin-2 (IL-2, also known as T-Cell Growth Factor, TCGF) is a lymphokine which is produced by lectin- or antigen-activated T-cells and plays an important immunoregulatory role. This factor, or lymphokine, was first identified by its ability to promote the long-term *in vitro* proliferation of activated T-cells. It also promotes the generation and proliferation of cytotoxic T-cells, natural killer (NK) cells, and lymphokine-activated killer (LAK) cells (1–11). Recombinant human IL-2 allows the cultivation of human and murine IL-2-dependent T-cell lines and natural killer cell lines, the proliferation of mitogenactivated T-lymphocytes and natural killer cells, the establishment of human and murine thymocyte-, splenocyte-, or peripheral blood lymphocyte (PBL)- derived T-cell lines, and the generation of human and murine lymphokine-activated killer (LAK) cells (1-11).

Preparation

Recombinant interleukin-2, human (hIL-2) is produced in *E. coli* and purified by standard chromatographic techniques.

Primary Structure

The primary structure of recombinant, IL-2, human is identical to that of natural, human IL-2 (one polypeptide chain, 133 amino acids), but recombinant IL-2 has an extra methionine at the amino-terminus (one polypeptide chain, 134 amino acids) and is not glycosylated (12-18). Glycosylation is not essential for biologicla activity.

Purity

≥95% pure as determined by SDS-PAGE [Endotoxin level: <0.1 EU/µg (LAL-test), <10 EU/ml (LAL-test].
1 EU corresponds to 0.1 ng.

Specific Activity/EC_{50}

$>2 \times 10^6$ U/mg (<0.5 ng/ml) [(hIL-2, NIBSC, 1st international standard, 86/504), at least the same specific activity (EC_{50}) compared to the indicated standard is guaranteed](19,20). Human, recombinant IL-2 has the same biological activity *in vitro* as compared to human, natural IL-2 (15, 1–4).

E_{50}Definition/Unit Definition

The amount of hIL-2 that is required to support half-maximal stimulation of cell proliferation (XTT cleavage) with CTLL-2 cells (1 unit equals ≤0.5 ng).

Molecular Weight

15,000 Da

Species Specificity

Recombinant IL-2, human is effective on mouse and human cells.

References

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** available from Roche Diagnostics*

5. Supplementary Information

Changes to Previous Version

- Editorial changes.

Text Conventions

To make information consistent and understandable, the following text conventions are used in this Instruction Manual:

Text Convention	Use
Numbered instructions labeled ❶, ❷ etc.	Steps in a procedure that must be performed in the order listed.

Symbols

Symbols are used in this Instruction Manual to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.

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