

For life science research only.  
Not for use in diagnostic procedures.



# Interleukin-2, human (hIL-2) recombinant (*E. coli*)

 **Version: 19**

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

**Store product at –15 to –25°C.**

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# 1. General Information

## 1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Catalog Number	Content
1	colorless	Interleukin-2, human (hIL-2)	<ul style="list-style-type: none"> <li>Solution, filtered through 0.2 µm pore size membrane.</li> <li>200 U/ml (0.1 µg/ml) in PBS (phosphate buffered saline) and 1 mg/ml BSA (bovine serum albumin).</li> </ul> <p><b>i</b> Purity of BSA: &gt;98%, endotoxin (LAL): &lt;1 EU/mg BSA.</p>	10 799 068 001	1 bottle, 50 ml
	red	Interleukin-2, human (hIL-2)	<ul style="list-style-type: none"> <li>Solution, filtered through 0.2 µm pore size membrane.</li> <li>10,000 U/ml (5 µg/ml) in PBS (phosphate buffered saline) and 1 mg/ml BSA (bovine serum albumin).</li> </ul> <p><b>i</b> Purity of BSA: &gt;98%, endotoxin (LAL): &lt;1 EU/mg BSA.</p>	11 011 456 001 11 147 528 001	1 bottle, 1 ml 1 bottle, 5 ml

## 1.2. Storage and Stability

### Storage Conditions (Product)

The product is shipped on dry ice.

When stored at –15 to –25°C, the product is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	colorless	Interleukin-2, human (hIL-2), recombinant ( <i>E. coli</i> ) 10,000 U (5 µg, 50 ml)	Store in aliquots at –15 to –25°C. <b>⚠️ Avoid repeated freezing and thawing.</b>
	red	Interleukin-2, human (hIL-2), recombinant ( <i>E. coli</i> ) 10,000 U (5 µg, 1 ml)	
	red	Interleukin-2, human (hIL-2), recombinant ( <i>E. coli</i> ) 50,000 U (25 µg, 5 ml)	

## 1.3. Additional Equipment and Reagent required

### Standard laboratory equipment

- 96 well tissue-culture grade, flat-bottomed microplates
- CO<sub>2</sub> incubator
- Centrifuge
- ELISA reader

### For the determination of hIL-2 activity

- Culture medium, for example, RPMI 1640, containing 10% FCS, 10 mM HEPES, 2 mM L-glutamine, 1x non-essential amino acids, 1 mM sodium pyruvate, and 50 µM 2-mercaptoethanol
- Cell Proliferation Kit II (XTT)\*

## 1.4. Application

Recombinant IL-2, human can be used for the:

- Cultivation of human and murine IL-2 dependent T-cell lines and natural killer cell lines.
- Proliferation of mitogen-activated T-lymphocytes and natural killer cells.
- Establishment of human and murine thymocyte, splenocyte, or peripheral blood lymphocyte (PBL)-derived T-cell lines.
- Generation of human and murine lymphokine-activated killer (LAK) cells.

## 2. How to Use this Product

### 2.1. Before you Begin

#### General Considerations

##### Primary structure

One polypeptide chain (133 amino acids) is identical to that of natural, human IL-2, however recombinant IL-2 has an extra methionine at the amino-terminus (one polypeptide chain, 134 amino acids) and is not glycosylated.

**i** *Glycosylation is not essential for biological activity.*

##### Working Solution

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

### 2.2. Protocols

#### Determination of hIL-2 activity on IL-2-dependent cells

The following steps were performed using CTLL-2 cells.

- 1 Prepare hIL-2 test samples as serial dilutions (final concentration 0.001 to 100 ng/ml) in culture medium in a 96-well, flat-bottomed microplate (tissue-culture grade) in a volume of 100  $\mu$ l.
 

**i** *As a negative control, use culture medium alone.*

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- 2 Harvest sensitive cells, such as CTLL-2 cells (ATCC TIB 214) and wash them three times by centrifugation in culture medium without hIL-2.
 

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- 3 Resuspend and adjust the cells in culture medium to  $4 \times 10^4$  cells/ml.
 

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- 4 Add 100  $\mu$ l of the cell suspension to 100  $\mu$ l of the prediluted hIL-2 samples into each well, revealing a final cell number of  $2 \times 10^4$  cells/ml ( $4 \times 10^3$  cells/well).
 

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- 5 Incubate the microplate for 48 hours at +37°C and 5% CO<sub>2</sub>.
 

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- 6 After the incubation period, add 100  $\mu$ l XTT labeling mixture to each well, see Instructions for Use for the Cell Proliferation Kit II (XTT)\*.
  - Incubate the microplate for another 6 hours at +37°C and 5% CO<sub>2</sub>.

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- 7 Measure the spectrophotometrical absorbance of the samples using a microplate (ELISA) reader, see section, **Results, Fig. 1**.
  - To measure absorbance of the formazan product, use a wavelength between 450 and 500 nm, for example 492 nm, depending on the optical filters available for the ELISA reader used.

**i** *Use a reference wavelength >650 nm.*

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## 2.3. Parameters

### Molecular Weight

15,000 Da

### Purity

≥95% pure as determined by SDS-PAGE.

Endotoxin level: ≤10 EU/ml (LAL).

**i** 1 EU corresponds to 0.1 ng.

### Specific Activity

≥2 MU/mg

(hIL-2, NIBSC, 1<sup>st</sup> international standard, 86/504); at least the same specific activity (EC<sub>50</sub>) compared to the indicated standard is guaranteed.

**i** Human, recombinant IL-2 has the same biological activity in vitro as compared to human, natural IL-2.

### Specificity

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

### Unit Definition

#### EC<sub>50</sub> 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).

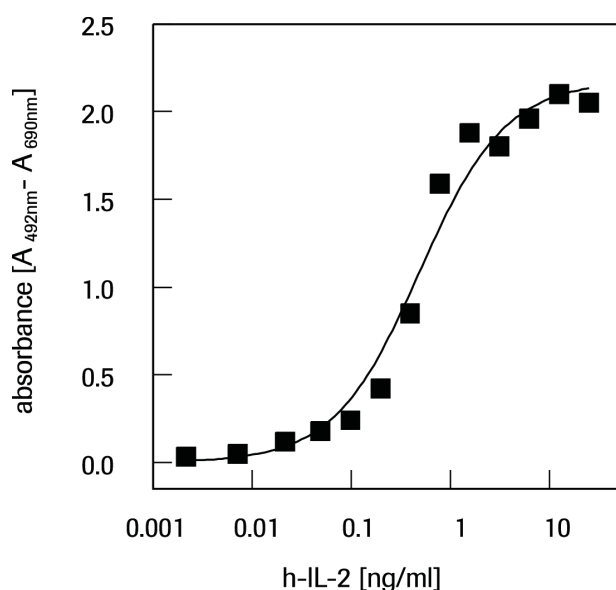
### Working Concentration

Established IL-2-dependent T-cell lines usually require 10 to 20 U/ml.

**i** Add IL-2 to the freezing medium for IL-2-dependent cell lines.

## 3. Results

### Absorbance of samples using an ELISA reader



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

## 4. Additional Information on this Product

### 4.1. Test Principle

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.

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 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.

### Preparation

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

### 4.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

## 5. Supplementary Information

### 5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

#### Text convention and symbols

**i** *Information Note: Additional information about the current topic or procedure.*

**⚠** **Important Note: Information critical to the success of the current procedure or use of the product.**

① ② ③ etc. Stages in a process that usually occur in the order listed.

① ② ③ etc. Steps in a procedure that must be performed in the order listed.

\* (Asterisk) The Asterisk denotes a product available from Roche Diagnostics.

### 5.2. Changes to previous version

Updated the Ordering Information.

### 5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Cell Proliferation Kit II (XTT)	1 kit, 2,500 tests	11 465 015 001



## 5.4. Trademarks

All product names and trademarks are the property of their respective owners.

## 5.5. License Disclaimer

For patent license limitations for individual products please refer to:

**Product Disclaimers.**

## 5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

## 5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

## 5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site.**

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

