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ab174444
IL-2 (Interleukin-2) Human SimpleStep ELISA® Kit

For the quantitative measurement of IL-2 in human cell culture supernatant, plasma and serum samples.

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1. Overview

IL-2 (Interleukin 2) in vitro SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of IL-2 protein in human cell culture supernatant, plasma and serum samples.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB Development Solution is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

IL-2, also known as T cell growth factor (TCGF), is a glycosylated alpha-helical polypeptide, synthesized as a 153 amino acid (aa) precursor with a 20 aa signal peptide and a 133 aa mature chain. It is secreted by activated CD4+ and CD8+ T cells, neurons, microglia and hematopoietic stem cells in response to antigenic or mitogenic stimulation. IL-2 is required for T-cell proliferation, Natural Killer cells (NK) cytolytic activity, differentiation of regulatory T cells, modulation of T helper (Th) cell differentiation and activation-induced cell death. In particular, IL-2 modulates the expression of receptors for other cytokines and transcription factors, therefore regulating cytokine cascades that correlate with each of the Th differentiation states.

Complete deficiency of IL-2 has been implicated in severe combined immunodeficiency, whereas reduction of the IL-2 correlates with reduced function of CD4+CD25+ regulatory T cells and destabilization of immune homeostasis leading to autoimmune disease. Increased

expression of IL-2 has also been implicated in inflammatory conditions such as inflammatory bowel disease and chronic liver diseases. IL-2 therefore is both a immune stimulator and immune suppressor cytokine which efficiently controls the immune system to deal with autoimmunity and adaptive immune response.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells

Add 50 µL Antibody Cocktail to all wells

Incubate at room temperature for 1 hour

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

Add 100 µL TMB Development Solution to each well and incubate for 5 minutes.

Add 100 µL Stop Solution and read OD at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
10X IL-2 Capture Antibody	600 µL	4°C
10X IL-2 Detector Antibody	600 µL	4°C
IL-2 Human Lyophilized Recombinant Protein	2 Vials	4°C
Antibody Diluent 4BI	6 mL	4°C
10X Wash Buffer PT	20 mL	4°C
TMB Development Solution	12 mL	4°C
Stop Solution	12 mL	4°C
Sample Diluent NS	50 mL	4°C
Sample Diluent 25BS	20 mL	4°C
Pre-Coated 96 Well Microplate (12 x 8 well strips)	96 Wells	4°C
Plate Seal	1	4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.4).
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- The provided 5X Cell Extraction Buffer contains phosphatase inhibitors. Protease inhibitors can be added if required.
- The provided 50X Cell Extraction Enhancer Solution may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The 50X Cell Extraction Enhancer Solution can be stored at room temperature to avoid precipitation.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. **The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.**
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Wash Buffer PT

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL 10X Wash Buffer PT with 45 mL deionized water. Mix thoroughly and gently.

9.2 Antibody Cocktail

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BI. Mix thoroughly and gently.

10. Standard Preparation

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

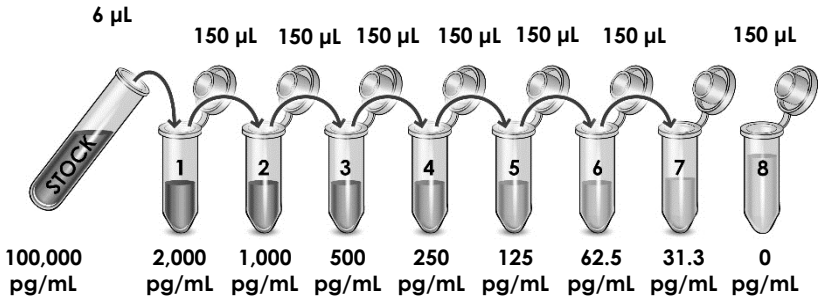
10.1 Reconstitute the IL-2 protein standard by adding 100 µL water. Mix thoroughly and gently. Hold at room temperature for 10 minutes and mix gently. Ensure all protein is reconstituted by inspecting against the light. This is the 100,000 pg/mL **Stock Standard** Solution.

10.2 Label eight tubes with numbers 1 – 8.

10.3 For **serum and plasma samples** add 294 µL Sample Diluent 25BS to tubes #1 and 150 µL Sample Diluent 25BS to tubes #2-8.

For **cell culture supernatant samples** add 294 µL Sample Diluent NS to tubes #1 and 150 µL Sample Diluent NS to tubes #2-8.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



11. Sample Preparation

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	**Range (%)**
48 hours PHA-stimulated PBMC supernatant	0.5 - 10%
Human Serum	1 - 50%
Human Plasma - Heparin	1 - 50%
Human Plasma - EDTA	1 - 50%
Human Plasma - Citrate	1- 50%

11.1. Plasma

Plasma samples must be diluted 2X with Sample Diluent NS prior to loading on the plate to ensure good linearity and recoverability of the protein. If greater dilution is desired, the Sample Diluent 25BS must be diluted by an equivalent factor in Sample Diluent NS before being used to dilute samples. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2. Serum

Serum samples must be diluted 2X with Sample Diluent NS prior to loading on the plate to ensure good linearity and recoverability of the protein. If greater dilution is desired, the Sample Diluent 25BS must be diluted by an equivalent factor in Sample Diluent NS before being used to dilute samples. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

11.3. Cell culture supernatant samples

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Cell culture supernatants should be diluted at

least ten fold in Sample Diluent NS to prevent saturation of the signal. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3 Add 50 µL of all samples and standards to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal or cover plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Development Solution to each well and incubate for 5 minutes in the dark (avoiding standard signal saturation) on a plate shaker set to 400 rpm.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed

time in the microplate reader prepared with the following settings:

Mode:	Kinetic
Wavelength:	600 nm
Time:	up to 15 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

13.9 Analyze the data as described below.

14. Calculations

14.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

14.2 Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

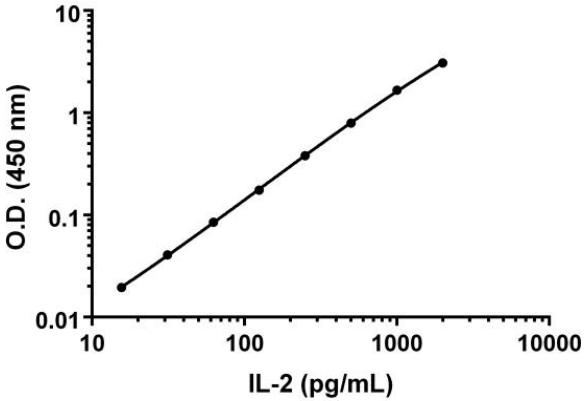
Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.4 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

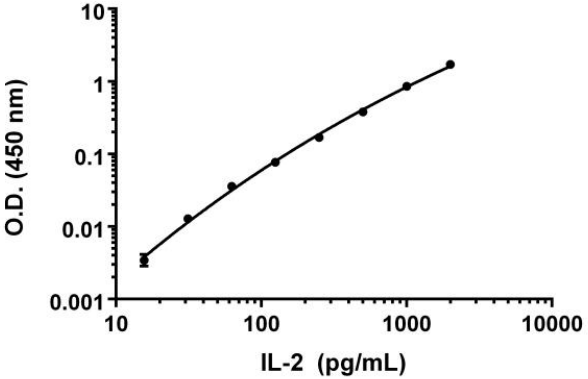
15. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.061	0.059	0.0602
15.63	0.088	0.087	0.088
31.3	0.112	0.106	0.109
62.5	0.164	0.162	0.163
125	0.285	0.246	0.265
250	0.556	0.482	0.519
500	1.081	0.908	0.995
1,000	1.938	2.014	1.976
2,000	3.559	3.548	3.553

Figure 1. Example IL-2 standard curve in Sample Diluents NS. The IL-2 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.058	0.057	0.058
15.63	0.061	0.061	0.061
31.3	0.069	0.072	0.070
62.5	0.093	0.094	0.093
125	0.136	0.133	0.134
250	0.232	0.222	0.227
500	0.439	0.433	0.436
1,000	0.912	0.914	0.913
2,000	1.720	1.811	1.765

Figure 2. Example IL-2 standard curve in Sample Diluent 25BS. The curve was prepared by loading standard curve as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Calibration

This immunoassay is calibrated against a highly purified mouse TNF alpha. The NIBSC/WHO unclassified purified mouse TNF alpha preparation 88/532 was evaluated in this kit.

The dose response curve of the unclassified standard 88/532 parallels the SimpleStep standard curve. To convert sample values obtained with the SimpleStep mouse TNF alpha kit to approximate NIBSC 88/532 units, use the equation below.

NIBSC (88/532) approximate value (units/mL) = 2.0 x SimpleStep mouse TNF alpha value (pg/mL)

17. Typical Sample Values

SENSITIVITY –

The calculated minimal detectable (MDD) dose is 9 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=24) and adding 2 standard deviations then extrapolating the corresponding concentrations.

Ten individual healthy donors were evaluated for the presence of IL-2 in serum using this assay. All samples measured less than the lowest IL-2 standard of 15.63 pg/mL.

RECOVERY –

(Sample spiking in representative sample matrices)

Sample Type	Average % Recovery	Range (%)
10% Culture Media	94	83 - 109
50% Human Serum	95	93 - 97
50% Human Plasma - Citrate	83	77 - 98
50% Human Plasma - Heparin	91	82 - 95
50% Human Plasma - EDTA	100	95 - 105

Linearity of Dilution

	Interpolated value	NHS 50%	NHP Citrate 50%	NHP EDTA 50%	NHP Heparin 50%	Media 10%
1:1	**pg/mL**	2,065	1,893	1,959	1,802	2,214
	% Expected value	**100**	**100**	**100**	**100**	**100**
1:2	**pg/mL**	1,010	964	964	920	1,061
	% Expected value	**102**	**102**	**98**	**102**	**105**
1:4	**pg/mL**	543	532	543	510	498
	% Expected value	**112**	**113**	**111**	**113**	**98**
1:8	**pg/mL**	293	293	305	281	243
	% Expected value	**124**	**125**	**125**	**124**	**94**

PRECISION –

Mean coefficient of variations of interpolated values from 2 concentrations of IL-2 protein within the working range of the assay.

	Intra-Assay	Inter-Assay
n =	6	24
CV(%)	6	6

SAMPLE VALUES –

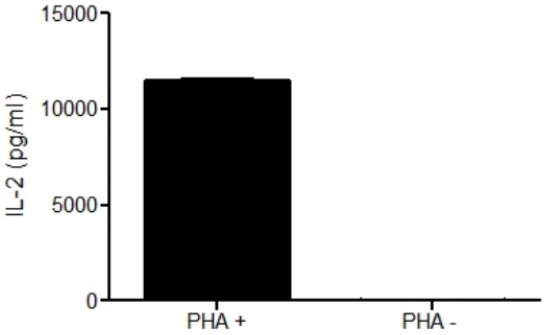


Figure 3. Specificity of IL-2 signal on stimulated and non stimulated media supernatants. Human PBMCs were cultured in RPMI supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were cultured for 2 days at 37^0C in the presence or absence of PHA. The concentrations of IL-2 were interpolated from the calibration curve and corrected for sample dilution. The mean IL-2 concentration was determined to be 16 pg/mL in unstimulated PBMC supernatants and 11,460 pg/mL in stimulated PBMC supernatants.

18. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB Development Solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

19. Notes

Technical Support

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