Human IL-2 ELISA

INTENDED USE: Enzyme Immunoassay based on microtiter plate for the detection and the quantitative determination of IL-2 in human plasma.

For professional use! Not for personal use! For performance evaluation only!

Cat.-No.: LIO-IL-2 01

INSTRUCTIONS Version: 2019-08-29

FEATURES:

Ready-to-use reagents
Microtiter strips
Detection via Peroxidase / TMB
Procedure time: appr. 230 minutes

Manufacturer / Hersteller:
LIONEX GmbH
Salzdahlumer Str. 196, Geb. 1A
D-38126 Braunschweig
Tel. +49-531-2601266
FAX +49-531-6180654

Distribution / Vertrieb:
LIONEX GmbH
Salzdahlumer Str. 196, Geb. 1A
D-38126 Braunschweig
Tel. +49-531-2601266
FAX +49-531-6180654

INTRODUCTION

Interleukin-2 (IL-2), so called T-cell growth factor (TCGF), is a peptide hormone out of the family of the interleukins. IL-2 was one of the first interleukins discovered [1, 2], the Glycoprotein is cloned and sequenced in 1983 first [6]. IL-2 is able to stimulate in-vitro growth of T-Lymphocytes from human bone marrow [3, 4], therefore it is used for therapy of cancer [5]. The protein binds to a specific IL-2 receptor consisting of 3 subunits (α, β and γ), which is mainly produced by T-cells. The β- and γ-Subunits (CD122 and CD132) are part of the cell membrane. Production of the α-Unit (CD25) is induced, if an antigen stimulates the corresponding T-cell. The IL-2 protein binds to the receptor, only if all 3 subunits are complete. After binding to the receptor a complex signal cascade for immune response is triggered. IL-2 binding to the specific receptor stimulates proliferation and differentiating of B- and T-lymphocytes, production of different other interleukins, interferon and tumor necrosis factors and cytotoxicity in activated macrophages [7, 8].

LIONEX has developed in-vitro-diagnostic ELISA test kits for the detection of Human-IL-2 in plasma samples. The LIONEX Human IL-2 ELISA test kit is suitable for rapid and reliable detection and quantitative determination of IL-2 in plasma. Lionex has also developed a Blood stimulating kit consisting of tubes for stimulating human blood samples called LIOKine®TB. IL-2 specific ELISA assays may help discriminating individuals with active or latent TB, if combined with the LIOKine®TB kit [9].

ADVANTAGES:

- ⇨ High sensitivity and specifity
- ⇨ High reproducibility
- ⇨ Minimal training necessary
- ⇨ Individual breakable wells
- ⇨ Quantitative results

KIT CONTENTS:

Σ 96 determinations, in-vitro-use only

MTP Microtiter plate coated with capture antibody (12 x 8 individual breakable wells, monoclonal anti human IL-2 antibody).

TMB TMB Substrate Solution: 12 ml, ready to use, contains a solution of tetramethylbenzidine (TMB).

STO Stop-Solution: 12 ml, ready to use, 0,2 M H_2SO_4

STA Recombinant Human IL-2 standard: 1 ng Human IL-2/vial, lyophilized, 2 vials.

IB Incubation buffer: 60 ml, ready to use, PBS/BSA buffer, addition of 0.05 % Tween-20 and 5-bromo-5-nitro-1,3-dioxane.

WB Wash Buffer, Concentrate (10 x): 100 ml, PBS buffer with Tween 20, addition of 0.05% 5-bromo-5-nitro-1,3-dioxane.

CON Conjugate solution, ready-to-use, addition of 0.05% 5-bromo-5-nitro-1,3-dioxane.

DET Detection antibody: monoclonal anti human IL-2-BIOTIN, ready-to-use, addition of 0.05% 5-bromo-5-nitro-1,3-dioxane.

One instruction manual.

Plastic Bag: Resealable, for the dry storage of unused strips.

STABILITY AND STORAGE CONDITIONS:

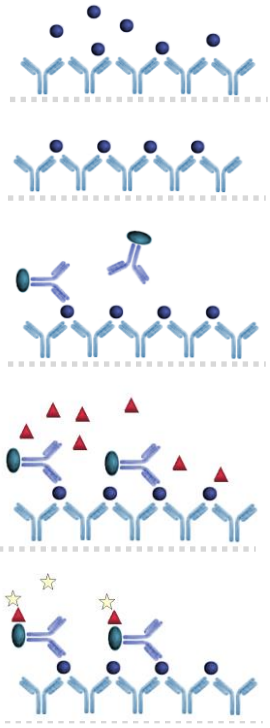
Unopened TEST KIT: until expiry date
Opened TEST KIT: components are stable for min. 2 month
Reconstituted standards: 48 h

2°C 8°C Perishable goods! Store at 2-8°C

MATERIALS NEEDED BUT NOT PROVIDED:

- 10 µl-, 100 µl- and 500 µl-micro- and multichannel pipets.
- Microtiter Plate-Reader (450 nm).
- Microtiter Plate-Washer (optional).
- Distilled water.
- Stop watch.
- Incubator (37°C).

PRINCIPLE OF THE TEST



Principle of IL-2 ELISA:

1) IL-2 is captured by anti-human IL-2 antibodies (immobilized on MTP)

2) Detection antibody (biotin-conjugated) attaches to the IL-2 captured.

3) Streptavidin-HRP (SA-HRP) attaches to the Biotin conjugated to the detection antibody.

4) HRP activity results in positive signal.

Figure 1: Principle of IL-2 ELISA.

The LIONEX Human IL-2 ELISA kit is based on the principle of the enzyme immunoassay (EIA, figure 1). Monoclonal Human IL-2 specific antibodies are bound on the surface of the microtiter strips as capture reagent. Plasma samples from human are pipetted into the wells of the microtiter plate in parallel to the standards. A binding between the IL-2 of the samples and the immobilized antibodies takes place. After 2 h incubation at room temperature the plate is rinsed with diluted wash solution, in order to remove unbound material. Then a biotin conjugated secondary antibody (detection antibody, ready-to-use) is pipetted into the wells and the microtiter plate is incubated for 60 min at room temperture. After a further washing step the ready-to-use peroxidase conjugate (Streptavidin-HRP) is added and incubated for additionally 60 min at room temperature. After a final washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes at room temperature, inducing the development of a blue dye in the wells. The colour development is terminated by the addition of a stop solution, which changes the colour from blue to yellow. The resulting dye is measured by an ELISA reader at the wavelength of 450 nm. The concentration of IL-2 is directly proportional to the intensity of the colour. Measuring each set of samples in parallel to the standard curve allows exact determination of the IL-2 concentration in each sample.

LIMITATIONS:

The test has been developed for the detection of human IL-2 in plasma. For detecting IL-2 in body fluids other than plasma the test has not been validated and can yield incorrect results!

We recommend using the LIOKine Blood cell stimulation kit for stimulating the samples (LIOKine®TB). Incubate samples accoding to the kit instructions. Pipet 1 mL each of the same sample into one tube containing PHA (positive control), one tube without protein (negative control) and one tube containing TB-antigens.

TB-diagnosis: the test is for evaluation purposes only and must not be used for TB-diagnosis.

The blood shall be kept at room temperature (15-30°C) after collecting and not used, if older than 7 h.

Only the clear supernatant shall be used for IL-2 measurements. While preparation of the samples avoid contamination by red blood cells. If necessary, separate the red blood cells from plasma by centrifugation. Avoid contamination of the samples!

PRECAUTIONS:

Follow the instructions and guidelines for test interpretation carefully!

1. Only for in-vitro use! Do not ingest or swallow! Do not eat, drink and smoke in the laboratory! Don't work without wearing protective clothing (gloves and lab coat)!
2. All kit components should be considered as infectious agents. Wipe off serum and reagent spills with a disinfecting solution (e.g. sodium hypochlorite, 5%)! Dispose residues of kit reagents and samples properly, e.g. by autoclaving.
3. Before use bring all reagents to room temperature (20-30 °C)!
4. Before pipetting, mix all reagents thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
5. Pipet with constant intervals, so that all the wells of the microtiter plate has the same conditions.
6. Avoid contamination of the reagents. Close bottles immediately after removing reagents to avoid oxidation.
7. Use separate disposable pipet tips.
8. Do not use reagents from different kit lots and do not mix reagents of different kits or kit lots with one another.
9. Use all reagents within the expiry period (mentioned on the kit label). After opening, the kit components are stable for 2 months.
10. In accordance with Good Laboratory Practice (GLP), all laboratory devices employed should be regularly checked for the accuracy and precision. This refers amongst others to microliter pipets, ELISA-Reader and washer.
11. Avoid the contact of kit reagents with skin, eye or mucosa, above all the stop-solution and the substrate.

PREPARATION OF REAGENTS

WB Wash Solution: If crystals precipitate during the cold storage, the concentrate should be warmed up at 37 °C for 15 minutes. Dilute 1:10 concentrate with distilled water before use (1 + 9 volume); e.g. 100 mL Wash buffer (10x concentrate) + 900 mL distilled water.

Samples and Sample Preparation:

Collect the samples using Heparin containing collectors (Lithium-Heparin or NH_4-Heparin).

Stimulating the samples by using the LIOKine®TB kit:
Transfer 1 mL of the sample collected into each of the LIOKine tubes (positive control = PHA; negative control = NIL, TEST). Place the tubes in cell incubator at 37°C for at least 48 h as indicated in the kit instructions of LIOKine®TB.

Remove the clear supernatant (plasma) from each tube and transfer to fresh tubes (e.g. Eppendorf vial).

Preparation of the standard curve:
Reconstitute lyophilized human IL-2 by addition of **1 mL** of incubation buffer to prepare a 1000 pg/mL stock solution.
Use the 1000 pg/mL stock solution to prepare further stock solutions by following the pipetting sheme below:

Stock solution:	conc. IL-2 [pg/mL]	Volume stock solution [µL]:	Volume Incubation buffer [µL]
1	400 pg/mL	400 µL	600 µL
2	200 pg/mL	500 µL of (1)	500 µL
3	100 pg/mL	500 µL of (2)	500 µL
4	50 pg/mL	500 µL of (3)	500 µL
5	25 pg/mL	500 µL of (4)	500 µL

For pipetting the reference curve use 25 – 400 pg/mL solutions!
The reconstituted standard (1000 pg/mL solution) is stabile at 2-8°C for minimum 4 days. Figure 2 shows how to prepare the standard curve.

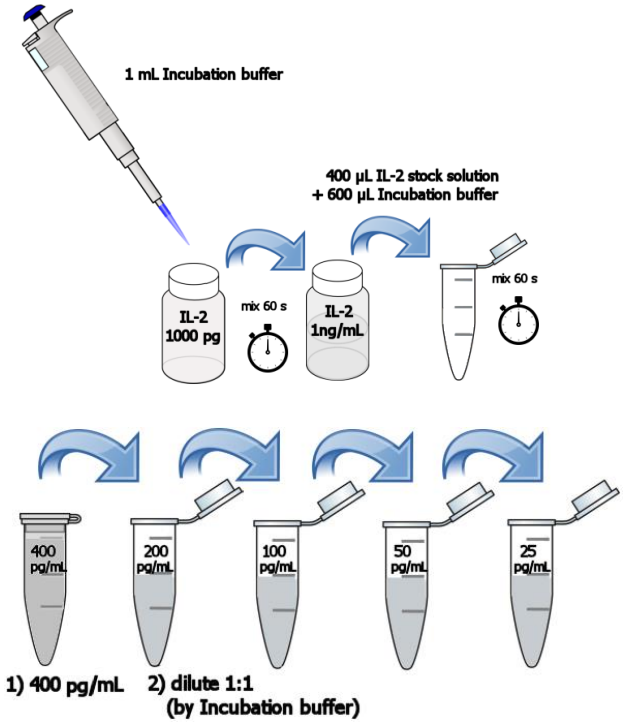


Figure 2: Reconstitution of the lyophilized standard and preparation of the standard curve by further dilution of the stock solution.

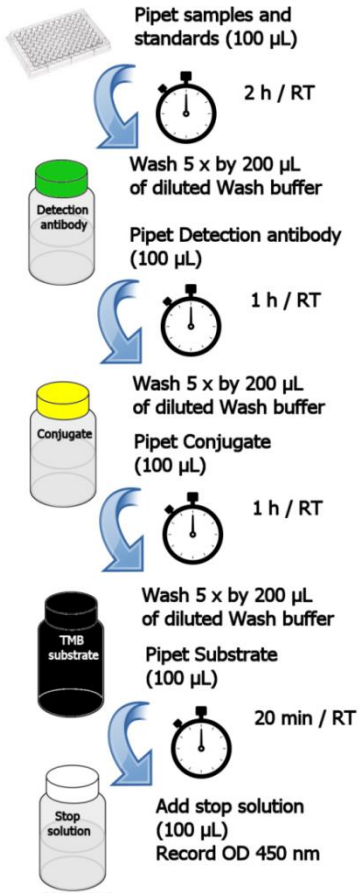
Attention: mix the liquids by shaking (e.g. Vortexer) for minimum 60 s! Mix the 1000 pg/mL stock solution after addition of the Incubation buffer for 60 s (Vortexer).

PROCEDURE:

1. Prepare test reagents: Equilibrate all kit components to room temperature. Dilute concentrated wash buffer 1:10 with distilled water. Reconstitute lyophilized IL-2 in Incubation buffer to a 1000 pg/mL stock solution by addition of 1 mL Incubation buffer. Prepare further stock solutions of IL-2 as described above.
2. Step A: Pipet 100 µl each of the samples and prepared standards STA (25 – 400 pg/mL) into the wells. All samples and standards should be measured in duplicate. Pipet Incubation buffer into well A1 and A2 for the substrate blank.
   INCUBATION: 2 h at 20-25°C.
3. Washing procedure: Empty the wells of the plate (dump or aspirate) and wash 5 x with diluted wash buffer WB (200 µl/well).
4. Step B: Pipet 100 µl each of the ready-to-use detection antibody DET into the wells.
   INCUBATION: 60 min at 20-25°C.
5. Washing procedure: Empty the wells of the plate (dump or aspirate) and wash 5 x with diluted wash buffer WB (200 µl/well).
6. Step C: Pipet 100 µl each of the ready-to-use conjugate CON into the wells.
   INCUBATION: 60 min at 20-25°C.
7. Washing procedure: Empty the wells of the plate (dump or aspirate) and wash 5 x with diluted wash buffer WB (200 µl/well).
8. Step E: Pipet 100 µl each of the ready-to-use substrate TMB into the wells.
   INCUBATION: 20 min at 20-25°C.
9. Step D: Terminate the substrate reaction: pipet rapidly 100 µl of the ready-to-use stop-solution STO into each well.
10. Measuring procedure: After gentle shaking, wipe the bottom of the plate and measure the absorption at 450 nm (optional reference wavelength: 620 nm). The colour is stable for at least 60 minutes.

Short reference guide of the test procedure:

See next page.



⇨ Calculate the concentration of each measured sample (in pg/mL) by using the equation of the reference curve:

Example of a REGRESSION EQUATION:
IL-2 [pg/mL](y) = (a • OD sample) + b
b = **-2,4472** / a = **316,13**

⇨ For exact determination of the IL-2 amount produced by blood cell stimulating for each antigen: subtract the value calculated for the NEGATIVE control from those observed for POSITIV control and TB-antigens.

Positive control:
conc. IL-2 produced [pg/mL] =
conc. IL-2 (PHA) – conc. IL-2 (NIL)

The value observed for the positive control shall be > 50 pg/mL.

TB-antigens:
conc. IL-2 produced [pg/mL] =
conc. IL-2 (TB-AG) – conc. IL-2 (NIL)

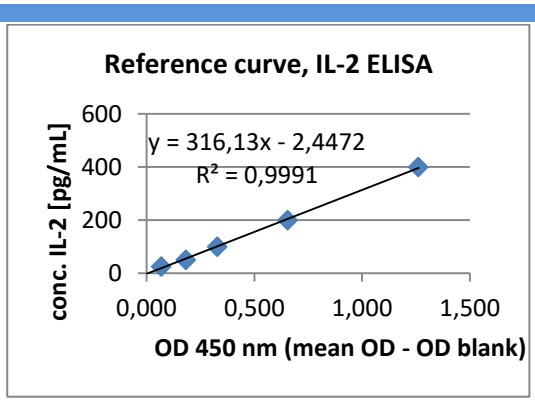


Figure 3: typical reference curve for Human IL-2 ELISA (LIO-IL-2 01).

CUT OFF LEVEL HUMAN IL-2 ELISA

Please use plasma samples of healthy and untreated TB-patients from the LIOKine®TB kit to determine the cut off value.

Restrictions: The reference curve should be a straight line. OD values for ready to use standards must be within the acceptable ranges defined in the kit inserts (± 10 %).

Note: if high background appears in a sample (OD > 0.5) dilute the sample by using incubation buffer (e.g. 1:5) and measure the same sample again!

SENSITIVITY AND SPECIFICITY:

Analytical sensitivity: detection limit of the kit is around 5 pg/mL.

Clinical sensitivity and specificity: studies for evaluating clinical sensitivity and specificity for TB are in progress.

INTERPRETATION OF RESULTS

Calculate the mean OD values for the measured absorptions for every sample. Substract the blank value from every calculated mean absorption: Mean OD sample – mean OD blank = OD 450

The difference between single values should not exceed 10 % for the standard solutions and samples.

QUANTITATIVE RESULTS

The values observed for the standards (reference curve) are used to calculate exact amounts of IL-2 in the samples. This results in reproducible quantitative evaluation. Consequently for a given patient, follow-up becomes possible.

REFERENCE CURVE: generating a scatter diagram with linear regression line:

For calculation of the reference curve it is recommended to use automatic computer programs.

⇨ Plot the OD results of each standard solution on the horizontal axis (x = OD 450) against the concentration of IL-2 of each standard on the vertical axis (y = conc. IL-2 [pg/mL]).

In the Scatter diagram with regression line, the relation between two variables is presented graphically and the regression line (the curve that fits best the plotted points) is drawn in the diagram. The equation of this curve is given in the regression window (figure 3). The resulting reference curve should be a straight line (R^2 > 0.980).

ASSAY PERFORMANCE

Precision: The intra- and inter assay coefficient of variation of the kit was assessed by a repeated determinations of a panel of samples. Intra- and Inter assay variation coefficient were below 10%.

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