RUO

ELISA-VIDITEST IL-2

REF OD-361

Instruction manual

1. TITLE

ELISA-VIDITEST IL-2 – ELISA kit for quantitative detection of interleukin 2

2. INTENTED USE

The ELISA-VIDITEST IL-2 kit is intended for determining the concentration of interleukin 2 in the test sample. Stimulation of leukocytes with specific antigen and subsequent measurement of the production of interleukin 2 is used as a proof of specific cellular immunity. The kit is intended only for research purposes.

3. TEST PRINCIPLE

ELISA-VIDITEST IL-2 assay is a solid-phase immunoanalytical test. It is based on the use of monoclonal antibodies that bind human interleukin-2 (IL-2) in the capture system. A specific anti-IL-2 antibody is immobilized on the surface of the wells in the microtiter plate. During the first incubation, molecules of IL-2 present in the test sasmple are bound to this antibody. A biotin-labeled anti-IL-2 detection antibody binds to them. The wells are then washed and incubated with conjugate (streptavidin-peroxidase). Binding of IL-2 to the detection antibody is then visualized by the enzymatic reaction of peroxidase with the chromogenic substrate. IL-2 concentration in the test sample is determined by a calibration curve prepared from the subsequent dilutions of the standard with defined concentrations of IL-2.

4. KIT COMPONENTS

ELISA break-away strips in a frame coated with specific antibody STRIPS	1x 12 pieces
2 mL lyophilized IL-2 standard ST IL-2 LYOF	2 vials
0.06 mL detection antibody, 101x conc. DET-AB 101x	1 vial
0.12 mL conjugate, 101x conc. CONJ 101x	1 vial
13 mL chromogenic substrate, r.t.u. [1] TMB-BF	1 vial
55 mL wash buffer, 10x conc. WASH 10x	1 vial
100 mL dilution buffer, r.t.u. DIL-TBS	1 vial
13 mL stop solution, r.t.u. STOP	1 vial

Instruction manual
Certificate of quality

[1] ready to use

Note: Control sera may be colorless to yellowish or blue due to the use of different diluents.

Chromogenic substrate TMB-BF r.t.u. is compatible and interchangeable between ELISA-VIDITEST kits, which contain TMB-BF, and is NOT RELIABLE with other TMB solutions used in other ELISA-VIDITEST TMB-O, TMB kits.

5. MATERIAL REQUIRED BUT NOT PROVIDED WITH THE KIT

Distilled/deionized water for dilution of the wash buffer concentrate, equipment for pipetting, solution dispensing and strip washing equipment, spectrophotometer/colorimeter for measuring the absorbance of the microtiter plate at 450 nm (reference filter 620-690 nm recommended), microplate shaker.

6. PREPARATION OF REAGENTS AND SAMPLES

a. **Allow all kit components to reach room temperature.**

b. Prepare **wash buffer** at working concentration by **diluting the buffer concentrate** **WASH** **10x** **10 times** with an appropriate volume of distilled/deionized water (e.g. 50 mL of the wash buffer + 450 mL of H_2O). If the salt crystals are in the concentrated solution, warm them in a water bath at 32-37 °C and mix thoroughly before diluting. Unused wash buffer at the working concentration can be stored in a closed container for 1 month at room temperature.

c. Prepare the **IL-2 Standard calibration curve:** Dissolve the lyophilized content of the vial ST IL-2 LYOF in 2 ml of dilution buffer DIL-TBS and mix gently (the concentration of dissolved standard is specified in the Certificate of quality for each lot). **Store the dissolved lyophilizate at +2 to + 10 °C. The expiration of the dissolved IL-2 standard is 1 week after reconstitution.** Pipette into a clean tube 0.2 mL of DIL-TBS and add 0.2 mL of dissolved lyophilizate (= Standard A, 2x dilution). Prepare another 5 dilutions of this Standard A as follows (binary dilution) - use a clean pipette tip for each dilution: Pipette into 5 clean tubes 0.2 mL of DIL-TBS. Add 0.2 mL of Standard A into the first tube and mix well (= Standard 1 (ST 1), 4x). Into the second tube add 0.2 mL of ST 1 and mix again (= Standard 2 (ST 2), 8x). Add 0.2 mL of ST 2 into the third tube and mix (= Standard 3 (ST 3), 16x), etc. This will provide a calibration curve of the five standards ST 1-ST 5.

d. Dilute the **detection antibody** **DET-AB** **101x** **101x with dilution buffer** DIL-TBS (e.g. add 50 µL of concentrated detection antibody to 5mL of dilution buffer and mix thoroughly). You will need 50 µL of detection antibody at working concentration per well.

e. **Just before use** dilute the **conjugate** **CONJ** **101x** **101x with dilution buffer** DIL-TBS (e.g. add 50 µL of the concentrated conjugate to 5mL of the dilution buffer and mix thoroughly). For one well, you will need 0.1 ml conjugate at working concentration.

f. Do not dilute other solutions, they are ready to use.

7. ASSAY PROCEDURE

Manufacturer will not be held responsible for results if manual is not followed exactly.

a. Allow strips, vacuum-sealed with desiccant, to reach room temperature before opening to avoid water condensation on the plate. Prepare the necessary number of strips for the reaction. Close the unused strips together with the desiccant well into the zip bag or seal them in a vacuum.

b. Fill the appropriate wells with 50 µL of **detection antibody** in the working concentration. Add in the first well 50 µL of **dilution buffer** **DIL-TBS** (= blank). Add 50 µL of prepared **standards** (ST 1-ST 5) to the next five wells - see the pipetting scheme. In the following wells pipette 50 µL of **sample**. Samples and standards can be applied in one well. To eliminate a laboratory error, apply samples and standards in two wells. Cover the plate with the lid and close it in a polyethylene zipper bag. Incubate **2 hours (+/- 5 min.) at room temperature (+20 - + 25°C) on the shaker.**

c. Aspirate contents of the wells into a safety collecting bottle containing appropriate disinfectant (see Safety Precautions). Wash the wells five times with 250 µL of wash buffer. Avoid overflowing the solution out of the wells . Aspirate the content of the wells and tap the plate on the cuttings of the cellulose wadding.

d. Pipette 100 µL of **conjugate** in the working concentration into all wells. Incubate **30 minutes (+/– 5 min.) at room temperature.**

e. Aspirate the liquid from the wells and wash them five times with 250 µl of wash buffer (see step c). Aspirate and tap.

f. Pipette 100 µL of **substrate solution** **TMB-BF** into all wells. Incubate **10 minutes (+/- 30 sec.) in the dark at room temperature.** Start measuring the incubation time after pipetting the first strip of

the plate. Follow this rule to avoid failure of time interval. Pipette fast in a regular rhythm or use a suitable dispenser. Cover the strips with an aluminum foil, an opaque lid, or keep it during reaction time in a dark place.

g. Stop the reaction by adding 100 µL **of stop solution STOP**. Pipette in the same rhythm as during filling of the substrate solution so that the enzymatic reaction takes place in all wells for the same time. Make sure there are no bubbles in the wells, if so, remove them by gently tapping the plate frame.

h. Measure the intensity of the colorimetric reaction on a spectrophotometer/colorimeter at 450 nm **within 10 minutes** after stopping the reaction. It is recommended to use reference filter at 620-690 nm.

Fig 1: Pipetting scheme

Strip nr.	1	2	3	4	5	6	7	8	9	10	11	12
A	DIL-TBS	S 3										
B	ST 1	S 4										
C	ST 2	S…										
D	ST 3											
E	ST 4											
F	ST 5											
G	S 1											
H	S 2											

8. PROCESSING OF THE RESULTS

Determine the concentration of IL-2 for the individual samples tested:

1. Deduct the absorbance value (OD) of the well with dilution buffer (DIL-TBS = background of the reaction) from the absorbance values of the samples and standards. If the absorbance of controls or tested sera are negative after background subtraction, consider them as zero value.

2. Construct the calibration curve by plotting the standard concentration on the x-axis (concentrations are listed in the Certificate of quality for each lot). On the Y-axis plot their absorbances (OD - DIL TBS).

3. Calculate the IL-2 concentration in samples from the equation of the calibration curve so that you substitute the OD value of the test sample (after subtracting the blank) instead of y-value. The x-values then represent the log of IL-2 concentration in the test sample. To calculate the concentrations of the IL-2 from calibration curve can be used suitable software, e.g. Winliana, Excel, or KIM-Q.

9. CHARACTERISTICS OF THE TEST

9.1. Validity of the test

Absorbance value (OD) of the blank (DIL-TBS) should be <0.250.

Absorbance values (OD) of the IL-2 standards should be in the range of conditions specified in the Certificate of quality for the given lot of the kit.

9.2. Precision of the test

To determine the variability between tests - reproducibility (inter-assay) and during testing – the repeatability (intra-assay) were compared with samples of different absorbance values.

9.2.1. Intra-assay variability

The variation coefficient of repeatability is max 15%. It is measured for each lot in a minimum of 8 parallel wells of the same plate.

An example (n= number of parallel wells in the same plate)

n	A	±σ	CV repeat
8	1.321	0.046	3.5 %
8	0.529	0.015	2.8 %

9.2.2. Inter-assay variability

The variation coefficient of reproducibility is max 15%. It is measured for each lot by comparing of wells with the same sample in several independent tests.

An example (n= number of tests of a particular sample)

n	A	±σ	min – max	CV repro
5	2.182	0.183	1.992 – 2.401	8.4 %
5	1.186	0.074	1.103 – 1.248	6.2 %
5	0.617	0.068	0.537 – 0.699	11.1 %
5	0.151	0.026	0.114 – 0.184	17.2 %

9.3. Detection limit

The detection limit for the IL-2 assay was determined using the WHO International Standard at 0.125 IU/mL.

10. INTERFERENCE

The culture medium for the stimulation of leukocytes (compl. RPMI1640 with 10% fetal bovine serum) did not have significant effect on the fixing of IL-2. Hemoglobin and lipids in a concentration of ≤ 100 mg/mL of blood have no IFNγ inhibitory effect in the ELISA. Bilirubin does not inhibit the test at concentration ≤ 10 mg/mL of blood.

11. SAFETY PRECAUTIONS

All ingredients of the kit are intended for laboratory use only.

With test specimens of culture medium from stimulated leukocytes, handle as infectious material and objects that have come into contact with them, autoclave for 1 hour at 121 °C, or disinfect for at least 30 minutes with a 3% solution of chloramine.

Liquid wastes containing Stop solution (sulfuric acid solution) before disposal neutralize in 4% sodium bicarbonate solution.

Work with Stop solution carefully to avoid staining of skin or mucous membranes. If it happens so, wash the affected area with plenty of running water.

Do not eat, drink or smoke while working. Do not pipette by mouth but with appropriate pipetting equipment. Wear protective gloves and thoroughly wash hands after work. Be careful not to spill samples and the aerosol formation.

12. HANDLING PRECAUTIONS

a. Avoid cross-contamination of reagents or contamination with substances that inhibit the enzymatic activity upon collection, dilution and storage of reagents.

b. The chromogenic substrate TMB-BF must not come into contact with oxidizing agents or metal surfaces.

c. Carefully follow the instructions in the Instruction manual. Non-reproducible results may arise in particular:

   * Insufficient mixing of reagents and samples before use
   * Inaccurate pipetting and inadequate incubation times stated in point 7.
   * Poor washing technique or spilling the rim of well with sample or reagents

* Use of identical pipette tip for different solutions or replacing closures.

13. STORAGE AND EXPIRATION

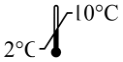
The ELISA kit should be used within three months after opening.

Store the kit and its components at +2 to +10 °C. Under these conditions, the expiration date of the kit is indicated on the central label on the package, the expiration of the individual components is indicated on its package. Do not freeze.

Put the unused strips back into the wrapper and seal them or close them well in the zipper bag together with the desiccant. Kits are transported in cooling bags, the transport time of up to 72 hours has no effect on expiration date. If you notice serious damage to the packaging of any part of the kit after receiving the kit, please inform the manufacturer immediately.

Solutions of test samples, diluted Standards, detection antibody and conjugate at working concentrations cannot be stored. Prepare it always fresh. Diluted wash solution can be stored in a sealed container for 1 month at room temperature.

14. USED SYMBOLS:

	number of wells		storage at +2°C - +10°C
	research use only	°C	Celsius degree
±σ	standard deviation	%	percentage
CV	coefficient of variation	n	number of tested samples
OD	optical density	A	value of tested sample
	manufacturer		consult instructions for use
	expiration date		catalogue code
	lot of kit		

References:

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15. FLOW CHART

Step 1	**Prepare reagents and samples in working dilution**
	↓
Step 2	**Dispense 50 µL of detection antibody and 50 µL of standards/samples per well**
	↓
	Incubate 2 hours at room temperature on shaker
	↓
Step 3	**Wash 5 times (250 µL/well), aspirate**
	↓
	Dispense 100 µL/well of conjugate
	↓
	Incubate 30 minutes at room temperature
	↓
Step 4	**Wash 5 times (250 µL/well), aspirate**
	↓
	Dispense 100 µL/well of TMB-BF substrate
	↓
	Incubate 10 minutes in the dark at room temperature
	↓
Step 5	**Dispense 100 µL/well of STOP solution**
	↓
Step 6	**Read the absorbance at 450/620-690 nm within 10 minutes**

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