

HUMAN INTERLEUKIN-27 ELISA

Product Data Sheet

Cat. No.: RGP018R

For Research Use Only

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➤➤ This kit is manufactured by:
BioVendor – Laboratorní medicína a.s.

➤➤ Use only the current version of Product Data Sheet enclosed with the kit!

1. INTENDED USE

The IL-27 ELISA is to be used for research use only quantitative determination of IL-27 in supernatants, buffered solutions, or serum and plasma samples. The assay will recognize both natural and recombinant IL-27. **This kit has been configured for research use only and is not to be used in diagnostic procedures.**

2. PRINCIPLE OF THE METHOD

The IL-27 Kit is a solid phase sandwich Enzyme Linked-Immuno- Sorbent Assay (ELISA). A monoclonal antibody specific for IL-27 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known IL-27 concentrations and unknowns are pipetted into these wells.

During the first incubation, IL-27 antigen is added to wells. After washing, a biotinylated monoclonal antibody specific for IL-27 is incubated. Then the enzyme (streptavidin-peroxydase) is added. After incubation and washing to remove all unbound enzyme, a substrate solution which actes on the bound enzyme is added to induce a coloured reaction product. The intensity of this coloured product is directly proportional to the concentration of IL-27 present in the samples.

3. INTRODUCTION

Interleukin IL-27 (IL-27) is a heterodimeric cytokine belonging to the IL-12 family that is composed of two subunits, Epstein-Barr virus-induced gene 3 (EBI3), a 33-kDa glycosylated protein, and IL-27p28, a 28-kDa peptide. IL-27 is an early product of activated antigen-presenting cells and drives rapid clonal expansion of naïve but not memory CD4⁺ T cells. The effects of IL-27 are eliciting by its interaction with a specific cell surface receptor complex composed of two proteins known as WSX-1 (TCCR) and gp130. IL-27 has both pro- and anti-inflammatory properties.

4. REAGENTS PROVIDED AND RECONSTITUTION

REAGENTS (Store at 2-8°C)	COLOUR CODE	Quantity	State
Antibody Coated Microtiter Strips		96 wells	Ready to use
Plastic cover		2	
Standard: 1000 pg/ml	Yellow	2 vials	Reconstitute with the volume of Standard diluent indicated on the vial. (See Reagents Preparation)
Standard Diluent buffer	Black	1 vial (30 ml)	Ready to use
Biotinylated anti IL-27	Red	1 vial (0.4 ml)	Dilute in biotinylated antibody diluent
Biotinylated Antibody Diluent	Red	1 vial (7.5 ml)	Ready to use
Streptavidin-HRP		2 vials (5 µl)	0.5 ml of HRP-Diluent before further dilutions
HRP Diluent	Red	1 vial (23 ml)	Ready to use
Washing Buffer	White	1 vial (10 ml)	200X concentrate. Dilute in distilled Water
Chromogen TMB :		1 vial (11 ml)	Ready to use
H ₂ SO ₄ : Stop Reagent	Black	1 vial (11 ml)	Ready to use

5. MATERIAL REQUIRED BUT NOT PROVIDED

- Distilled water.
- Pipettes : 10 µl, 50 µl, 100 µl, 200 µl and 1000 µl.
- Vortex mixer and magnetic stirrer.

6. SAFETY

- For research use only.
- Handling of reagents, serum or plasma specimens should be in accordance with local safety procedures , e.g. CDC/NIH Health manual : " Biosafety in Microbiological and Biomedical Laboratories" 1984.
- Avoid any skin contact with H₂SO₄ and TMB. In case of contact, wash thoroughly with water.
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
- Do not pipette by mouth.

7. PROCEDURAL NOTES/LAB. QUALITY CONTROL

1. When not in use, kit components should be stored refrigerated or frozen as indicated on vials or bottles labels. All reagents should be warmed to room temperature before use. Lyophilized standards should be discarded after use.
2. Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration.
3. Cover or cap all reagents when not in use.
4. Do not mix or interchange reagents between different lots.
5. Do not use reagents beyond the expiration date of the kit.
6. Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid crosscontamination; for the dispensing of H₂SO₄ and substrate solution, avoid pipettes with metal parts.
7. Use a clean plastic container to prepare the washing solution.
8. Thoroughly mix the reagents and samples before use by agitation or swirling.
9. All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
10. The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent colour development. Warning TMB is toxic avoid direct contact with hands. Dispose off properly. If a dark blue colour develops, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbances within 1 hour after completion of the assay.
11. When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
12. Respect incubation times described in the assay procedure.
13. Dispense the TMB solution within 15 min. following the washing of the microtiter plate.

8. SPECIMEN COLLECTION, PROCESSING AND STORAGE

Cell culture supernatants - Remove particulates and aggregates by spinning at approximately 1000 x g for 10 min.

Serum – Use pyrogen/endotoxin free collecting tubes. Serum should be removed rapidly and carefully from the red cells after clotting. For that, after clotting, centrifuge at approximately 1000 x g for 10 min and remove serum.

Storage - If not analyzed shortly after collection, samples should be aliquoted (250-500 µl) to avoid freeze-thaw cycles and stored frozen at –70°C. Avoid multiple freeze-thaw cycles of frozen specimens. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particles are present, this should be removed prior to assay by centrifugation or filtration.

Recommendation: Do not thaw by heating at 37°C or 56°C. Thaw at room temperature and make sure that sample is completely thawed and homogeneous before assaying.

9. PREPARATION OF REAGENTS

8.1 Standards

Standard vials have to be reconstituted with the volume of Standard diluent indicated on the vial. This reconstitution gives a stock solution of 1000 pg/ml IL-27. Serial dilutions of standard must be made before each assay and cannot be stored.

8.4 Dilution of biotinylated anti IL-27

Extemporaneous preparations are recommended. Dilute the biotinylated anti IL-27 with the biotinylated antibody diluent in a clean glass vial according to the number of wells to be used. See the next table for volumes to pipette.

Number of Wells used	Biotinylated Antibody (µl)	Biotinylated Antibody Diluent (µl)
16	40	1060
24	60	1590
32	80	2120
48	120	3180
96	240	6360

8.5 Dilution of Streptavidin-HRP

Add 0.5 ml of HRP diluent to a 5 µl vial of Streptavidin-HRP . DO NOT KEEP THIS DILUTION FOR FURTHER EXPERIMENTS. Dilute immediately before use. Following the number of wells to be used, further dilutions of Streptavidin-HRP should be made with HRP diluent in a clean glass vial : see hereafter the table for volumes to pipette.

Number of Wells	Streptavidin-HRP(µl)	Strep-HRP Diluent (ml)
16	30	2
24	45	3
32	60	4
48	75	5
96	150	10

8.6 Washing Buffer 200X concentrate

Dilute 200 times in distilled water.

10. ASSAY METHOD

- a) Before use, mix all reagents thoroughly without making foam.
- b) Determine the number of microwell strips required to test the desired number of samples, plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and samples should be assayed **in duplicate**. Remove sufficient microwell strips from the pouch.
- c) Add 100 µl of standard diluent to standard wells B1, B2, C1, C2, D1, D2, E1, E2, F1, F2. Reconstitute standard vial with the appropriate volume as described in the chapter reagents preparation. Pipet 200 µl of standard into wells A1 and A2 (see Plate Scheme below). Transfer 100 µl from A1 and A2 to B1 and B2 wells. Mix the contents by repeated aspirations and ejections. Take care not to scratch the inner surface of microwells. Repeat this procedure from the wells B1, B2 to wells C1, C2 and from wells C1, C2 to D1, D2 and so on creating two parallel rows of IL-27 standard dilutions ranging from 1000 to 31.25 pg/ml. Discard 100 µl from the content of the last microwells used (F1, F2). Alternatively these dilutions can be done in separate tubes and diluted standard pipetted directly into wells.
- d) Add 100 µl of standard diluent to the blank wells (G1-G2) and 100 µl of sample to sample wells.
- e) Cover with a plate cover and incubate for 2 hours at room temperature (18°C - 25°C).
- f) Remove the cover and wash the plate as follows:
 - 1) aspirate the liquid from each well;
 - 2) dispense 0.3 ml of washing solution into each well;
 - 3) aspirate again the content of each well;
 - 4) Repeat steps 2) and 3) two times.
- g) Preparation of biotinylated anti IL-27: (see preparation of reagents).

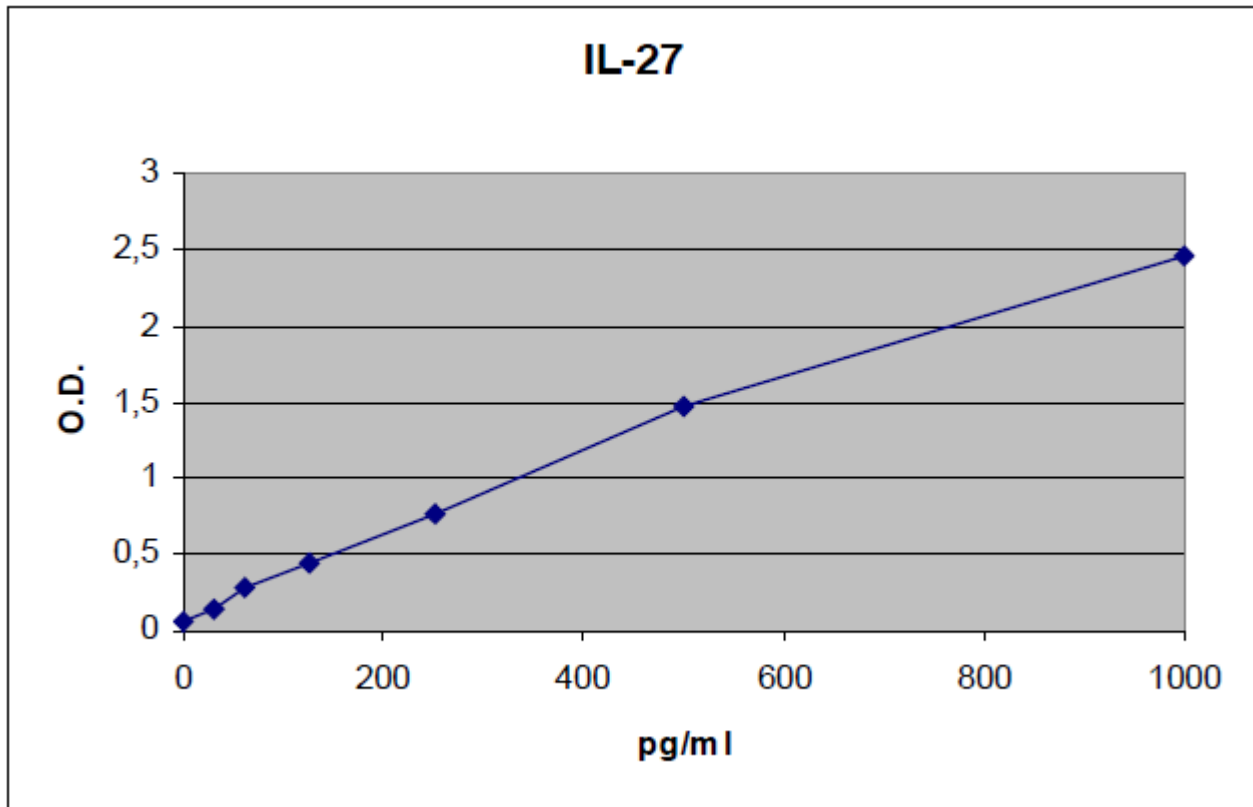
- h) Add 50 µl of diluted biotinylated anti IL-27 to all wells.
- i) Cover and incubate 1 hour at room temperature.
- j) Wash as described in point f)
- k) Prepare HRP solution just before use: (see preparation of reagents).
- l) Dispense 100 µl of HRP solution into all wells, including the blank wells. Put back the cover.
- m) Incubate the microwell strips at room temperature for 30 minutes.
- n) Remove plate cover and empty wells. Wash microwell strips according to point (f). Proceed immediately to the next step.
- o) Pipette 100 µl of ready-to-use TMB substrate solution into all wells, including the blank wells and incubate in the dark for 5-15 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
- p) Incubation time of the substrate solution is usually determined by the ELISA reader performances: many ELISA readers record absorbance only up to 2.0 O.D. *The O.D. values of the plate should be monitored and the substrate reaction stopped before positive wells are no longer properly readable.*
- q) The enzyme-substrate reaction is stopped by quickly pipetting 100 µl of H₂SO₄: stop reagent into each well, including the blank wells, to completely and uniformly inactivate the enzyme. Results must be read rapidly after the addition of H₂SO₄: stop reagent.
- r) Read absorbance of each well on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm (610 nm to 650 nm is acceptable) as the reference wavelength.

11. SUGGESTED PLATE SCHEME

		Sample wells											
Standard Concentrations pg/mL		1	2	3	4	5	6	7	8	9	10	11	12
A	1000	1000											
B	500	500											
C	250	250											
D	125	125											
E	62,5	62,5											
F	31,25	31,25											
G	Blank	Blank											
H													

12. DATA ANALYSIS

Generate a linear standard curve by plotting the average absorbance on the vertical axis versus the corresponding IL-27 standard concentration on the horizontal axis. The amount of IL-27 in each sample is determined by extrapolating OD values to IL-27 concentrations using the standard curve.



Typical IL-27 standard curve ranging from 1000 to 31.25 pg/mL

13. LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 1000 pg/ml standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples (> 1000 pg/ml) have to be diluted with Standard diluent or with your own sample buffer. During analysis, multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced...) has not been investigated. The rate of degradation of native IL-27 in various matrices has not been investigated.

14. PERFORMANCES AND CHARACTERISTICS

13.1 Sensitivity

The minimum detectable dose of IL-27 is less than 12.8 pg/ml.

This has been determined by adding 3 standard deviations to the mean optical density obtained when the zero standard was assayed 30 times.

13.2 Specificity

The assay recognizes both natural and recombinant human IL-27. To define specificity of this ELISA, several proteins were tested for cross reactivity. There was no cross reactivity observed for any protein tested (IL-6, IL-6R, gp130, IL-10, IL-12, IL-12p40, IL23, ICAM-1).

13.3 Spiking - Recovery

The spiking recovery was evaluated by spiking three concentrations of IL-27 in human serum in two experiments. **Recoveries ranged from 82 to 103 % with an overall mean recovery of 95 %.**

13.4 Precision

Intra-Assay					Inter-Assay				
Sample	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
A	9	343	12,9	4,8	A	9	343	29	8,5
B	9	127	8,1	5,1	B	9	157	17	10,8
C	9	427	15,8	3,8	C	9	427	38	9

13.5 Linearity of Dilution

Four spiked human serum with different levels of IL-27 were analysed at three serial two fold dilutions (1:2-1:8) with two replicates each. Recoveries ranged from 103% to 118% with an overall mean recovery of 110%.

13.6 Storage Stability

Aliquots of spiked serum samples were stored at -20°C , $2-8^{\circ}\text{C}$, room temperature (RT) and at 37°C and the IL-27 level determined after 24h. We observed a loss of 15% after storage at RT and 35% after storage at 37°C .

13.7 Freeze-thaw stability

Aliquots of spiked serum were stored frozen at -20°C and thawed up to 5 times and IL-27 level was determined. We observed a loss of 10%.

13.8 Expected values

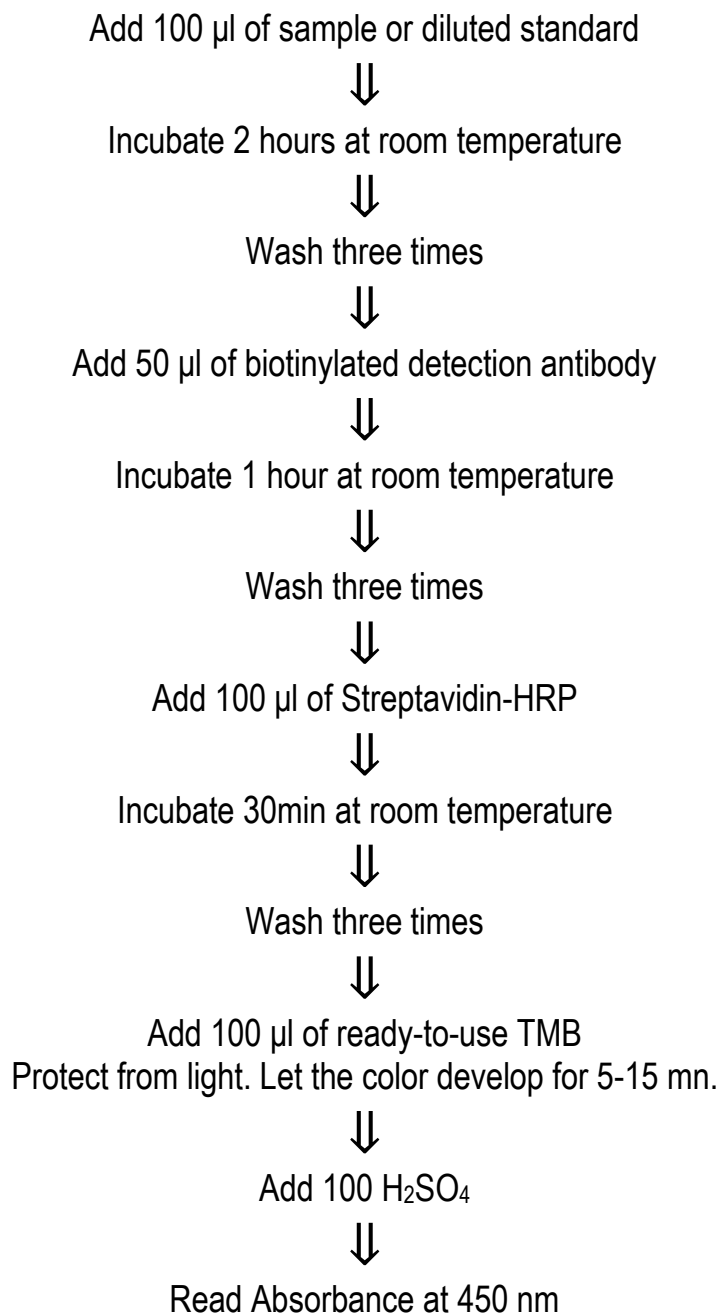
A panel of 40 apparently healthy blood donors was tested for IL-27. The detected level of IL-27 ranged from 0 (for 38 sera) and 135 pg/ml (1 serum).

15. REFERENCES

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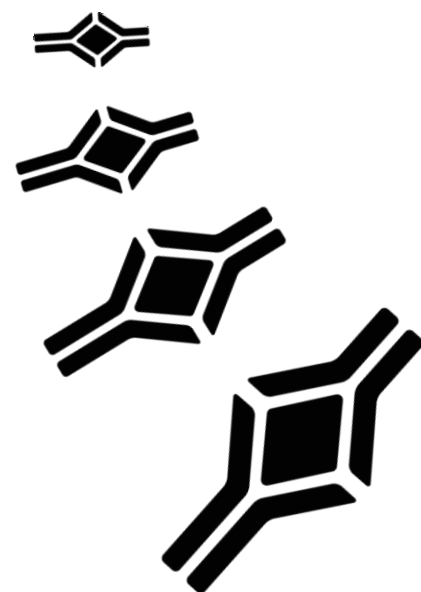
16. ASSAY PROCEDURE SUMMARY

Total procedure length 3 h 45 mn



NOTES





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