IL27/IL27B (Human) ELISA Kit

Catalog Number KA 2215

96 assays

Version: 03

Intended for research use only

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Introduction

Intended Use

Detection and Quantification of Human Interleukin-27 (hIL-27) Concentrations in Cell Lysates, Sera and Plasma.

Background

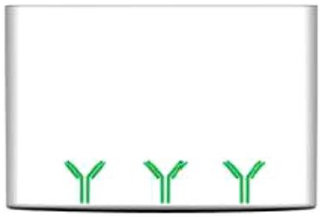
IL-27 is a cytokine with pro- and anti-inflammatory properties that can regulate T_H development, suppress T-cell proliferation, stimulate T_C activity, induce isotype switching in B-cells, and has diverse effects on innate immune cells. Among its target cells are CD4 T_H which can differentiate in type 1 effector cells (TH1), type 2 effector cells (TH2) and IL17 producing helper T-cells (TH17). In addition, it drives rapid clonal expansion of naive but not memory CD4 T-cells. IL-27 also strongly synergizes with IL-12 to trigger IFN-gamma production of naive CD4 T-cells, bind to the cytokine receptor WSX-1/TCCR which appears to be required but not sufficient for IL-27-mediated signal transduction. IL-27 potentiates the early phase of TH1 response and suppresses TH2 and TH17 differentiation while it induces the differentiation of TH1 cells via two distinct pathways, p38 MAPK/TBX21- and ICAM1/ITGAL/ERK-dependent pathways. This protein also induces STAT1, STAT3, STAT4 and STAT5 phosphorylation and activates TBX21/T-Bet via STAT1 with resulting IL12RB2 up-regulation, an event crucial to TH1 cell commitment. It suppresses the expression of GATA3, the inhibitor TH1 cells development. In CD8 T-cells, it activates STATs as well as GZMB.
IL-27 reveals to be a potent inhibitor of TH17 cell development and of IL-17 production. Indeed IL-27 subunit p28 alone is also able to inhibit the production of IL17 by CD4 and CD8 T-cells. While IL-27 suppressed the development of proinflammatory Th17 cells via STAT1, it inhibits the development of anti-inflammatory inducible regulatory T-cells, iTreg, independently of STAT1. IL-27 has also an effect on cytokine production, suppressing proinflammatory cytokine production such as IL2, IL4, IL5 and IL6 and activating suppressors of cytokine signaling such as SOCS1 and SOCS3. Moreover, IL-27 antagonizes the effects of some cytokines such as IL6 through direct effects on T cells. Another important role of IL-27 is its antitumor activity as well as activation of production of anti-angiogenic chemokines such as IP-10/CXC10 and MIG/CXCL9. In vein endothelial cells, it induces IRF1 and increase the expression of MHC class II transactivator/CIITA with resulting up-regulation of major histocompatibility complex class II. IL-27 also demonstrates antiviral activity with inhibitory properties on HIV-1 replication.

Principle of the Assay

The IL27/IL27B (Human) ELISA Kit contains the components necessary for quantitative determination of natural or recombinant hIL-27 concentrations within any experimental sample including cell lysates, serum and plasma. This particular immunoassay utilizes the quantitative technique of a “Sandwich” Enzyme-Linked Immunosorbent Assay (ELISA) where the target protein (antigen) is bound in a “sandwich” format by the

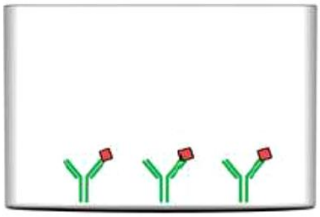
primary capture antibodies coated to each well-bottom and the secondary detection antibodies added subsequently by the investigator. The capture antibodies coated to the bottom of each well are specific for a particular epitope on the Human IL-27 cytokine while the user-added detection antibodies bind to epitopes on the captured target protein. Amid each step of the procedure, a series of wash steps must be performed to ensure the elimination of non-specific binding between proteins to other proteins or to the solid phase. After incubation and “sandwiching” of the target antigen, a peroxidase enzyme is conjugated to the constant heavy chain of the secondary antibody (either covalently or via Avidin/Streptavidin-Biotin interactions), allowing for a colorimetric reaction to ensue upon substrate addition. When the substrate TMB (3, 3’, 5, 5’-Tetramethylbenzidine) is added, the reaction catalyzed by peroxidase yields a blue color that is representative of the antigen concentration. Upon sufficient color development, the reaction can be terminated through addition of Stop Solution (2 N Sulfuric Acid) where the color of the solution will turn yellow. The absorbance of each well can then be read by a spectrophotometer, allowing for generation of a standard curve and subsequent determination of protein concentration.

Capture Antibody



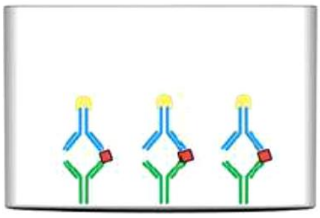
Monoclonal capture antibodies specific for the target are adsorbed to the plate.

Target Antigen



Target antigen present in standard or sample is bound by capture antibodies on the solid-phase.

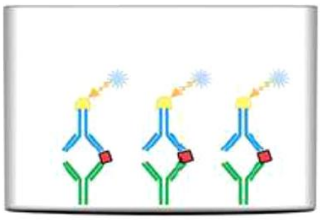
Biotin-Labeled
Detection Antibody



Polyclonal, biotin-labeled detection antibodies specific for the target are added to bind another epitope on the target antigen.

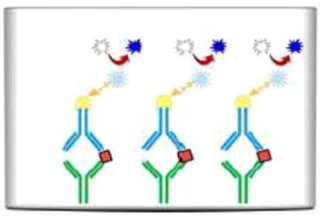
Avidin-HRP
Enzyme Conjugate

Unreacted TMB



Avidin-HRP attaches to detection antibody via high affinity avidin-biotin interaction.

Blue TMB
Diimine Product



TMB substrate is converted to the blue TMB diimine via the HRP enzyme. Upon addition of acid, the reaction terminates and the wells can be read at 450 nm.

General Information

Materials Supplied

List of component

Reagent	Quantity Per Plate	Container	Reconstitution
96-Well Microplate or Strips Coated w/ Capture Antibody	12 x 8 Strips	-	-
Ready-to-Use Streptavidin-HRP	12 ml	Clear	-
Ready-to-Use Substrate	12 ml	Brown	-
Stop Solution	12 ml	Clear	-
Wash Buffer (10X)	50 ml	Clear	-
Protein Standard Diluent	12 ml	Clear	-
Sample Diluent	12 ml	Clear	-
Detection Antibody Diluent	12 ml	Clear	-
Biotin-Labeled Detection Antibody	Lyophilized	Yellow	100 µl H_2O
Protein Standard	Lyophilized (5.3 ng)	Red	50 µl H_2O
Adhesive Plate Sealers	4 Sheets	-	-

Storage Instruction

- ✓ *Note: If used frequently, reagents may be stored at 4°C.*
- ✓ Unopened Kits: Store at 4°C for 6 months.

Component	Storage Time	Storage Information
96-Well Microplate or Strips Coated w/ Capture Antibody	6 months	4°C
Ready-to-Use Streptavidin-HRP		
Ready-to-Use Substrate		
Stop Solution		
Wash Buffer (10X)		
Protein Standard Diluent		
Sample Diluent		
Detection Antibody Diluent		
Protein Standard	Lyophilized: 6 months	4°C
Biotin-Labeled Detection Antibody	Reconstituted: 1 month	
Adhesive Plate Sealers	-	-

Materials Required but Not Supplied

The following materials and equipment are NOT provided in this kit but are necessary to successfully conduct the experiment:

- ✓ Microplate reader able to measure absorbance at 450 nm (with correction wavelength set to 540 nm or 570 nm)
- ✓ Micropipettes with capability of measuring volumes ranging from 1 µl to 1 ml
- ✓ Deionized or sterile water
- ✓ Squirt bottle, manifold dispenser, multichannel pipette reservoir or automated microplate washer
- ✓ Graph paper or computer software capable of generating or displaying logarithmic functions
- ✓ Absorbent paper or vacuum aspirator
- ✓ Test tubes or microfuge tubes capable of storing ≥1 ml
- ✓ Bench-top centrifuge (optional)
- ✓ Bench-top vortex (optional)
- ✓ Orbital shaker (optional)

Precautions for Use

- ✓ Assay Restrictions

- This ELISA kit is intended for research purposes only, NOT diagnostic or clinical procedures of any kind.
- Materials included in this kit should NOT be used past the expiration date on the kit label.
- Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits.
- Variations in pipetting technique, washing technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in binding affinity of the materials provided.
- The assay is designed to eliminate interference and background by other cellular macromolecules or factors present within any biological samples. However, the possibility of background noise cannot be fully excluded until all factors have been tested using the assay kit.

- ✓ Health and safety Precautions

- Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment.
- Stop Solution contains 2 N Sulfuric Acid (H_2SO_4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.

Assay Protocol

Reagent Preparation

- ✓ Reconstitute the Biotin-Conjugated Detection Antibody in 100 µl of ddH_2O for a concentration of 72 µg/ml.
- ✓ Reconstitute the Protein Standard in 50 µl of ddH_2O for a concentration of 160 ng/ml
- ✓ Diluent the 50 ml of 10 x Wash Buffer in 450 ml of ddH_2O for 500 ml of 1x Wash Buffer

Sample Preparation

If samples are to be used within 24 hours, aliquot and store at 4°C. If samples are to be used over a long period of time, aliquot and store between -20°C and -80°C, depending on the duration of storage.

Note: Samples containing a visible precipitate or pellet must be clarified prior to use in the assay.

Caution: Avoid repeated freeze/thaw cycles to prevent loss of biological activity of proteins in experimental samples.

- ✓ Cell Lysate and Supernatants

  Remove large cell components via centrifugation and perform the assay. Cell lysates and supernatants require a dilution using Sample Diluent. A serial dilution may be performed to determine a suitable dilution factor for the sample. For future use of the sample, follow the sample storage guidelines stated above.
- ✓ Serum

  Allow samples to clot in a serum separator tube (SST) for 30 minutes. After sufficient clotting, centrifuge at 1000 x g for 15 minutes and remove serum from SST in preparation for the assay. Serum samples require at least a 1: 50 dilution using Sample Diluent. For future use of the sample, follow the storage guidelines above.
- ✓ Plasma

  Use heparin, citrate or EDTA as an anticoagulant to gather plasma from original biological sample. After collection of the plasma, centrifuge for 15 minutes at 1000 x g. This step must be performed within 30 minutes of plasma collection. Plasma samples require at least a 1:50 dilution using Sample Diluent. Afterwards, perform the assay or for future use of the sample, follow the storage guidelines stated above.

Assay Procedure

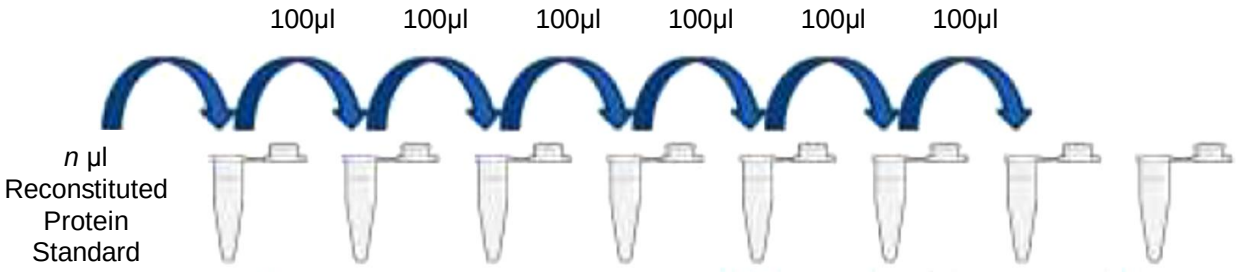
- ✓ *Note: If possible, all incubation steps should be performed on an orbital shaker to equilibrate solutions when added to the microplate wells. Also, all provided solutions should be at ambient temperature prior to use.*
- ✓ *Note: Avoid adding solutions into wells at an angle, always keep pipette tip perpendicular to plate bottom.*

✓ Addition of Known Standard and Unknown Sample to Immunoassay

The IL27/IL27B (Human) ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Human IL-27 proteins within the range of 157-10000 pg/ml.

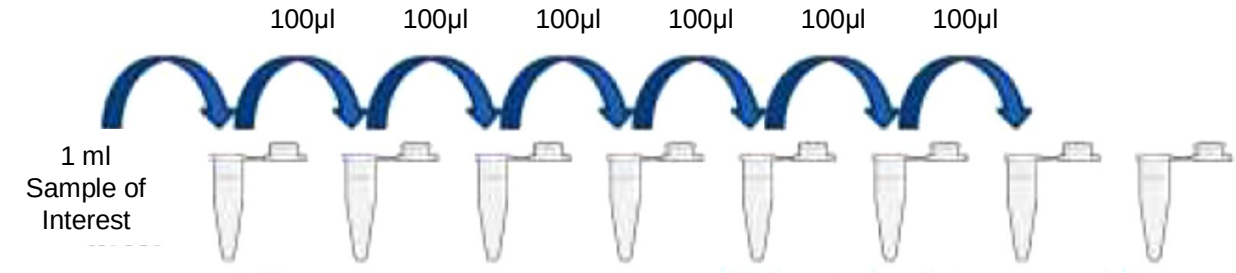
- Dilute the known standard sample from 10000 pg/ml to 0 ng/ml in a series of microfuge tubes. Mix each tube thoroughly by inverting several times or by vortexing lightly to ensure proper equilibration. Add 100 µl of each serial dilution step into the wells of a specified row or column of the 96-well microtiter plate in duplicate or triplicate and incubate at room temperature for 2 hours. Unknown sample of interest can be serial diluted with Sample Diluent to concentrations within the detection range of this assay kit and added to the plate at 100 µl per well. Seal the microplate air-tight using one of the microplate adhesive seals provided in this kit or Parafilm if readily available. See Plate Layout for serial dilution diagram.

To obtain serial dilution high point, dilute reconstituted Protein Standard to the maximum concentration for serial dilution by adding n µl reconstituted Protein Standard to serial dilution high point tube and then raising the volume to 200 µl. Shown below is a diagram illustrating a hypothetical 2-fold serial dilution on a given reconstituted Protein Standard.



	High Point	1:2 Dilution	1:4 Dilution	1:8 Dilution	1:16 Dilution	1:32 Dilution	1:64 Dilution	Negative Control
Starting:	200µl	100µl	100µl	100µl	100µl	100µl	100µl	100µl
Remaining:	100µl	100µl	100µl	100µl	100µl	100µl	200µl	100µl

For samples of unknown protein concentrations, serial dilute the experimental sample using Sample Diluent to determine range of detection and acceptable dilutions. Shown below is a diagram illustrating a 10-fold serial dilution on a given Sample of Interest.



	Sample of Interest	1:10 Dilution	1:100 Dilution	1:1000 Dilution	1:10000 Dilution	1:00000 Dilution	1:000000 Dilution	Negative Control
Starting:	1000µl	900µl	900µl	900µl	900µl	900µl	900µl	100µl
Remaining:	900µl	900µl	900µl	900µl	900µl	900µl	1000µl	100µl

✓ Addition of Detection Antibody to Capture Antibody-Bound Samples

1. Aspirate the protein standard solution out of the microplate wells. If your lab does not have a vacuum-based aspirator, you may dump the solutions from the microplate into a waste container and blot 3-4 times on a stack of paper towels until most or all of the liquid is removed from the wells. Dilute the 10X wash buffer to 1X using pure H_2O. Add 300-400 µl of Wash Buffer to each well being used and gently shake for 5-7 minutes on an orbital shaker. Perform this wash step 4 times consecutively.
2. After the 4th wash step, dilute the detection antibody solution 1:180 in detection antibody diluent to a concentration of 400 ng/ml. Mix the test tube either by inverting several times or vortexing to ensure proper equilibration. Ensure that there is enough detection antibody solution for all wells being used. Add 100 µl of the diluted detection antibody solution into each well, seal the plate and incubate at room temperature for 2 hours.

✓ Conjugation of Streptavidin-HRP with Biotin-Labeled Detection Antibody

1. Remove the detection antibody solution out of the microplate wells by either vacuum-based aspirator or paper towel blotting. Perform 4 consecutive wash steps with gentle shaking between each wash.
2. After the 4^{th} wash step, add 100 µl of Ready-To-Use Streptavidin-HRP Conjugate Solution into each well and incubate at room temperature for 30 minutes.

✓ Application of Liquid Substrate for Colorimetric Reaction

1. Remove the Streptavidin-HRP conjugate solution out of the microplate wells by either vacuum-based aspirator or paper towel blotting. Prepare the ready-to-use substrate solution by bringing it to room temperature without exposure to fluorescent or UV light as these may degrade the substrate. Perform 4 consecutive wash steps with gentle shaking between each wash.
2. After the 4^{th} wash step, add 100 µl of ready-to-use substrate solution into each well and incubate at room temperature for color development. The microplate should be kept out of direct light by either covering with an opaque object or putting it into a dark room. Closely monitor the color development as some wells may turn blue very quickly depending on analyte and/or detection antibody-HRP concentrations. Once the blue color has ceased to develop further, immediately add 100 µl of Stop Solution to each well being used. The color in the wells should immediately change from blue to yellow.
3. The microplate is now ready to be read by a microplate reader. Within 30 minutes of adding the Stop Solution, determine the optical density (absorbance) of each well by reading the plate with the microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm.
   Caution: Readings made directly at 450 nm without correction may be higher and less accurate.

✓ Summary

1. Reconstitute Biotin-Conjugated Detection Antibody and Protein Standard and dilute the 10x Wash Buffer as specified.
2. Perform serial dilution of Protein Standard and prepare samples as desired. See sample preparation section for instructions to dilute serum and plasma samples.
3. Add 100 µl of Standard, sample or control to each well and incubate for 2 hours at room temperature.
4. Aspirate Standards, samples or controls out and wash plate 4 times.
5. Dilute Biotin-Conjugated Detection Antibody as specified. Add 100 µl to each well and incubate for 2 hours at room temperature.
6. Aspirate Biotin-Conjugated Detection Antibody out and wash plate 4 times.
7. Add 100 µl of Ready-to Use Streptavidin-HRP to each well and incubate at room temperature for 30 minutes.
8. Aspirate Ready-to Use Avidin-HRP out and wash plate 4 times.
9. Add 100 µl of Ready-to Use Substrate to each well and incubate at room temperature for color development.
10. Add 100 µl of Stop Solution and read plate at 450 nm.

Data Analysis

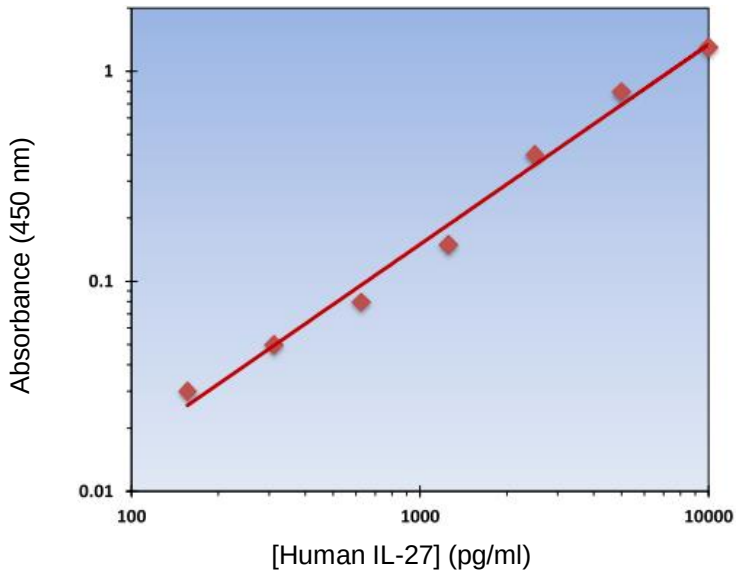
Calculation of Results

✓ Generation of Standard Curve and Interpretation of Data

1. Average the duplicate or triplicate readings for each standard, control and sample and subtract the average zero standard optical density.
2. Generate a standard curve by using Microsoft Excel or other computer software capable of establishing a 4-Parameter Logistic (4-PL) curve fit. If using Excel or an alternative graphing tool, plot the average optical density values in absorbance units (y-axis) against the known standard concentrations in pg/ml (x-axis). *Note: Only use the values in which a noticeable gradient can be established. Afterwards, generate a best fit curve or "trend-line" through the plotted points via regression analysis. Note: Shown on the next page is an example of typical data produced by analysis of the standard sample.*

The data and subsequent graph was obtained after performing a cytokine ELISA for Human IL-27. Each known sample concentration was assayed in triplicate.

Human IL-27 Standard Curve	
Concentration (pg/ml)	Average OD 450nm
10000	1.3
5000	0.8
2500	0.4
1250	0.15
625	0.08
312.5	0.05
156.25	0.03



Performance Characteristics

✓ Sensitivity

The IL27/IL27B (Human) ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Human IL-27 proteins within the range of 157-10000 pg/ml.

✓ Cross Reactivity and Specificity

- The IL27/IL27B (Human) ELISA Kit is capable of recognizing both recombinant and naturally produced Human IL-27 proteins. The antigens listed below were tested at 50 ng/ml and did not exhibit significant cross reactivity or interference.
  Human: IL-12, IL-12/IL-23 p40
  Murine: IL-27

Resources

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard (High Point)	Standard (High Point)	Standard (High Point)	sample	sample	sample	sample	sample	sample	sample	sample	sample
B	Standard (1:2)	Standard (1:2)	Standard (1:2)	sample	sample	sample	sample	sample	sample	sample	sample	sample
C	Standard (1:4)	Standard (1:4)	Standard (1:4)	sample	sample	sample	sample	sample	sample	sample	sample	sample
D	Standard (1:8)	Standard (1:8)	Standard (1:8)	sample	sample	sample	sample	sample	sample	sample	sample	sample
E	Standard (1:16)	Standard (1:16)	Standard (1:16)	sample	sample	sample	sample	sample	sample	sample	sample	sample
F	Standard (1:32)	Standard (1:32)	Standard (1:32)	sample	sample	sample	sample	sample	sample	sample	sample	sample
G	Standard (1:64)	Standard (1:64)	Standard (1:64)	sample	sample	sample	sample	sample	sample	sample	sample	sample
H	Negative Control	Negative Control	Negative Control	sample	sample	sample	sample	sample	sample	sample	sample	sample