picoIL-6 ELISA

AL-1007-r

RUO

INTENDED USE

The PicoIL-6 enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of IL-6 in serum and other biological fluids. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.

SUMMARY AND EXPLANATION

Interleukin-6 (IL-6) is a member of the IL-6 superfamily, which consists of polypeptide cytokines with a four-α-helix structure and a molecular mass of 21 to 28 kDa. It is produced by the lymphoid and non-lymphoid cells with pleotropic functions ranging from hematopoiesis and metabolic regulation to inflammation, autoimmunity, and acute phase response.

Under normal conditions, IL-6 plays an important role in cellular homeostasis. However, during inflammatory conditions, the concentration of IL-6 increases in several folds. This relates its clinical relevance as a major alarm signal in humans in response to infections (sepsis/septicemia), inflammation, autoimmunity, and cancer.[1-3] Increased concentrations of IL-6 have been reported in localized (prosthetic joint infections, periodontitis), and systemic (eg, sepsis, coronavirus 2019 [COVID-19]) infections, autoimmune conditions (eg, rheumatoid arthritis (RA), systemic lupus erythematosus, ankylosing spondylitis, and inflammatory bowel disease), and cancers. [3-7] Overall, IL-6 cytokine is nonspecific biomarker of systemic inflammation that may have relevance in clinical decision making in the appropriate context.

PRINCIPLE OF THE TEST

The picoIL-6 ELISA is a quantitative two-step immunoassay. In the first step, Calibrators, Controls and unknown diluted samples are added to IL-6 capture antibody coated microtiter wells along with the biotinylated detection antibody solution and incubated. After the first incubation, and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP) solution. After the third incubation and washing step, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution. In principle, the antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of IL-6 in the samples and calibrators.

MATERIALS SUPPLIED

CAL-1007A - CAL-1007F IL-6 Calibrators A-F (Lyophilized)

Six vials, labeled A-F, containing concentrations of approximately 0-125 pg/mL IL-6 in protein-based buffer with non-mercury preservative. Refer to **calibration card** for exact concentrations. Store unopened vial at 2 to 8°C until the expiration date. Reconstitute calibrators B-F with 1 mL deionized water. Solubilize, mix well, and use after reconstitution. Aliquot and freeze at 20°C or colder for up to one year. Avoid repeated freeze thaws. Discard after 5 days, if stored at 2 to 8°C.

Note: *The IL-6 calibrators are traceable to the WHO International Standard, NIBSC Code: 89/548, Version 4.0.*

CTR-1007-I and CTR-1007-II IL-6 Controls (Lyophilized)

Two vials, labeled Levels I and II containing low and high IL-6 in protein-based buffer with a non-mercury preservative. Refer to **calibration card** for exact control ranges. Store unopened at 2 to 8°C until the expiration date. Reconstitute control Levels I and II with 1 mL deionized water. Solubilize, mix well, and use after reconstitution. Aliquot and freeze at 20°C or colder for up to one year. Avoid repeated freeze thaws. Discard after 5 days, if stored at 2 to 8°C.

PLT-1007 IL-6 Antibody Coated Microtitration Strips

One strip holder, containing 96 polystyrene microtitration wells with IL-6 antibody immobilized to the inside wall of each well. Store at 2 to 8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

CND-1007 IL-6 Conjugate Diluent

One bottle, 12 mL, containing a protein-based buffer with a non-mercury preservative. Store at 2 to 8°C until expiration date.

BCC-1007 IL-6 Biotin Conjugate Concentrate

One vial, 0.4 mL, containing IL-6 antibody conjugated to HRP in a protein buffer with a non-mercury preservative. Store at 2 to 8°C until the expiration date.

SAR-1007 IL-6 Streptavidin Conjugate Ready-To-Use

One bottle, 12 mL, containing streptavidin-HRP (horseradish peroxidase) in a protein-based buffer and a non-mercury preservative. Store undiluted at 2-8°C until expiration date.

TMB-100 TMB Chromogen Solution

One bottle, 12 mL, containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2 to 8°C until expiration date.

WSH-100 Wash Concentrate A

One bottle, 60 mL, containing buffered saline with a nonionic detergent. Store at 2 to 30°C until expiration date. Dilute 25-fold with deionized water prior to use.

STP-100 Stopping Solution

One bottle, 12 mL, containing 0.2 M sulfuric acid. Store at 2 to 30°C until expiration date.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microtitration plate reader capable of absorbance measurement at 450 nm, 405nm and 630 nm.
2. Microtitration orbital plate shaker.
3. Microtitration plate washer.
4. Semi-automated/manual precision pipette to deliver 10–250 µL.
5. Vortex mixer.
6. Deionized water.
7. Disposable 12 x 75 mm culture tubes.
8. Tight fitting 12 x 75 mm tube racks.

WARNINGS AND PRECAUTIONS

For Research Use Only. Not for use in diagnostic procedures. The following precautions should be observed:

a) Follow good laboratory practice.

b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.

c) Handle and dispose of all reagents and material in compliance with applicable regulations

WARNING: Potential Biohazardous Material

This reagent may contain some human source material (e.g. serum) or materials used in conjunction with human source materials. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 5th Edition, 2007[8].

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 as a preservative. Pro-Clean 400 and peroxide in concentrated amounts are irritants to skin and mucous membranes.

For further information regarding hazardous substances in the kit, please refer to the MSDS, either at AnshLabs.com or by request.

SAMPLE COLLECTION AND PREPARATION

a. Sample handling, processing, and storage requirements depend on the brand of blood collection tube that you use. Please refer to the manufacturer's instructions for guidance. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products.

b. Samples must be stored at 2°-20°C to avoid loss of bioactivity and contamination.

c. Avoid assaying lipemic, hemolyzed or icteric samples.

d. Avoid repeated freezing and thawing of samples. Thaw samples no more than 3 times.

e. For shipping, place specimens in leak proof containers in biohazard specimen bags with appropriate specimen identification and test requisition information in the outside pocket of the biohazard specimen bag. Follow DOT and IATA requirements when shipping specimens.[9]

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the picoIL-6 ELISA assay. It is the responsibility of the customer to validate the assay for their use. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.
2. A calibration curve must be included with each assay.
3. Bring all kit reagents to room temperature before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix different lots of any kit component and do not use any component beyond the expiration date.
4. Use a clean disposable pipette tip for each reagent, calibrator, control, or sample. Avoid microbial contamination of reagents, contamination of the substrate solutions with the HRP conjugates. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use deionized water.
5. Incomplete washing will adversely affect the outcome and assay precision. Care should be taken to add TMB into the wells accurately and efficiently to minimize potential assay drift due to variation in the TMB incubation time. Avoid exposure of the reagents to excessive heat or direct sunlight.

PREPARATION OF REAGENTS

1. **IL-6 Calibrators A-F and IL-6 Controls I & II**: Tap and reconstitute IL-6 Calibrator A-F and IL-6 Controls I & II each with 1 mL deionized water. Solubilize, mix well and use after reconstitution.
2. **Wash Solution:** Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature when stored in a tightly sealed bottle.
3. **IL-6 Antibody-Biotin Conjugate Solution:** The IL-6 Antibody-Biotin Conjugate Concentrate should be diluted at a ratio of 1 part into 50 parts of the IL-6 conjugate diluent, according to the number of wells used. For an entire plate, pipet exactly 120 µL of the Antibody-Biotin Conjugate Concentrate into 6 mL of the IL-6 Conjugate Diluent.
   NOTE: *The antibody-biotin conjugate concentrate should be freshly diluted 10–15 minutes prior to use.*
4. **Microtitration Wells:** Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

ASSAY PROCEDURE

Allow all samples and reagents to reach room temperature. Mix reagents thoroughly by gentle inversion before use. After reconstitution of reagents, mix thoroughly, avoiding foam. Calibrators, controls, and samples should be assayed in duplicate.

1. Reconstitute IL-6 Calibrator A-F and IL-6 Controls I & II each with 1 mL deionized water. Solubilize for **10 minutes**, Mix well.
2. Mark the microtitration strips to be used.
3. Prepare the Biotin Conjugate Solution by diluting the Antibody-Biotin Conjugate Concentrate with the IL-6 Conjugate Diluent as described under the "Preparation of Reagents" section of this package insert.
4. Pipet **50 µL** of the calibrators, controls, and unknown samples to the appropriate wells.
5. Add **50 µL** of the Antibody-Biotin Conjugate Solution (Prepared in Step 3) to each well using a repeater pipette.
6. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for **1 hour** at room temperature (23 ± 2°C).
7. Aspirate and wash each well **5 times** with the wash solution **(350 µL/per well)** using an automatic microplate washer.
8. Add **100 µL** of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
9. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 minutes** at room temperature (23 ± 2°C).
10. Aspirate and wash each strip **5 times** with the Wash Solution **(350 µL/per well**) using an automatic microplate washer.
11. Add **100 µL** of the TMB chromogen solution to each well using a repeater pipette. Avoid direct exposure to heat and sunlight.
12. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for **8-10 min** at room temperature (23 ± 2°C).
    NOTE: *Visually monitor the color development to optimize the incubation time.*
13. Add **100 µL** of the stopping solution to each well using a repeater pipette.
14. Read the absorbance of the solution in the wells within 20 minutes, using a microplate reader set to 450 nm.
    NOTE**: Zero calibrator should be programmed as "**Blank**" while reading the optical density. If instrument has a wavelength correction, set the instrument to dual wavelength measurement at **450 nm** with background wavelength correction at **630 nm.

RESULTS

***NOTE:** The results in this package insert were calculated by plotting the **log optical density (OD) data on the y-axis and log IL-6 concentration on X-axis** using a cubic regression curve-fit. Alternatively, log vs. log*

quadratic regression curve-fit can be used. Other data reduction methods may give slightly different results.

1. Optimum results can be obtained at incubation temperature of **23 ± 2°C**.
2. Determine the IL-6 concentrations of the controls and samples from the calibration curve by matching their mean OD readings with the corresponding IL-6 concentrations.
3. Any sample reading higher than the highest calibrator should be appropriately diluted using calibrator A and re-assayed.
4. Any sample reading lower than the analytical sensitivity should be reported as such.

LIMITATIONS

The reagents supplied in this kit are optimized to measure IL-6 levels in human serum and other biological fluids. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the samples[10].

QUALITY CONTROL

a. Each laboratory should establish mean values and acceptable ranges to assure proper performance.
b. IL-6 ELISA controls or other commercial controls should fall within established confidence limits.
c. The confidence limits for picoIL-6 ELISA controls are printed on the calibration card.
d. A full calibration curve, low- and high-level controls, should be included in each assay.
e. The TMB chromogen solution should be colorless. Development of a blue color may indicate reagent contamination or instability.

REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Well Contents Calibrators	Mean Absorbance	Conc (pg/mL)
A1, A2	A	0.092 (Blank)	0
B1, B2	B	0.052	1
C1, C2	C	0.11	2.5
D1, D2	D	0.47	10
E1, E2	E	2.36	60
F1, F2	F	3.77	125

CAUTION: *The above data must not be employed in lieu of data obtained by the user in the laboratory.*

ANALYTICAL CHARACTERISTICS

All analytical characteristics are stated in pg/mL.

Analytical Sensitivity:

The analytical sensitivity in the assay as calculated by the interpolation of mean plus two standard deviation of 18 replicates of calibrator A (0 pg/mL) and calibrator B (1.0 pg/mL) is 0.15 pg/mL.

Imprecision:

Reproducibility of the IL-6 ELISA assay was determined in a study using three serum pools. The study included a total of 5 assays, three replicates of each per assay (n=15). Representative data were calculated and are presented in the following table.

Sample	Mean conc.	Within run		Between run		Total	
	(pg/mL)	SD	%CV	SD	%CV	SD	%CV
Sample 1	6.1	0.3	4.1%	0.4	6.7%	0.5	7.8%
Sample 2	45.7	2.7	6.0%	0.5	1.2%	2.8	6.1%
Sample 3	78.2	5.5	7.1%	0	0.0%	5.5	7.1%

Linearity:

Based on dilutions of the four serum samples containing various IL-6 levels diluted with Calibrator A the % recovery on individual samples is represented in the following:

Sample	Dilution Factor	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
1	Neat	81.85		
	1:2	40.92	37.66	92%
	1:4	20.46	18.92	92%
	1:8	10.23	9.55	93%
2	Neat	50.73		
	1:2	25.37	25.47	100%
	1:4	12.68	12.58	99%
	1:8	6.34	6.35	100%
3	Neat	8.19		
	1:2	4.09	4.27	104%
	1:4	2.05	2.23	109%
	1:8	1.02	0.98	95%
4	Neat	42.7		
	1:2	21.35	20.3	95%
	1:4	10.67	10.26	96%
	1:8	5.34	4.89	92%

Recovery:

Known amounts of IL-6 were added to three serum samples containing different levels of endogenous IL-6. The concentration of IL-6 was determined before and after the addition of exogenous IL-6 and the percent recovery was calculated.

Sample	Endogenous Conc.(pg/mL)	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
1	0.8	27.74	27.4	99%
		43.91	44.16	101%
		54.68	54.53	100%
2	10.84	19.94	18.1	91%
		25.4	23.34	92%
		29.04	26.48	91%
3	0.91	12.38	12.19	99%
		19.26	19.22	100%
		23.84	23.26	98%

Analytical Specificity:

The monoclonal antibody pair used in the assay does not cross-react to other closely related analytes. The antibody pair used in picoIL-6 assay detects human, mouse, rabbit serum samples. The assay does not detect Bovine, Canine, Equine, Ovine, Porcine and Goat serum samples.

Cross-Reactant	Concentration	% Cross reactivity
IL-1, 92/644	50,000 pg/mL	Non-Detectable
IL-2, 86/500	15300 pg/mL	Non-Detectable
IL-3, 91/510	50,000 pg/mL	Non-Detectable
IL-4, 88/656	50,000 pg/mL	Non-Detectable
IL-8, 89/520	50,000 pg/mL	Non-Detectable
TNF-α, 12/154	50,000 pg/mL	Non-Detectable

Interference:

When potential interferents (hemoglobin, biotin, intralipids and bilirubin) were added at least at two times their physiological concentration to control sample, IL-6 concentration was within ± 10% of the control as represented in the following table.

Interferent	Interferent Dose	Sample IL-6 (pg/mL)	Dosed Sample IL-6 (pg/mL)	% Difference to Reference
Hemoglobin	1 mg/mL	20.10	19.90	-1.2
	0.5 mg/mL	20.50	21.30	4.0
	0.1 mg/mL	21.60	21.80	1.0
Hemoglobin	1 mg/mL	83.80	88.50	5.5
	0.5 mg/mL	94.60	101.50	7.3
	0.1 mg/mL	98.90	100.50	1.6
Biotin	1200 ng/mL	20.90	20.90	0.0
	600 ng/mL	21.70	21.10	-2.7
	200 ng/mL	21.10	22.50	6.4
Biotin	1200 ng/mL	84.90	91.50	7.8
	600 ng/mL	97.30	98.40	1.1
	200 ng/mL	104.10	202.70	-1.3
Intralipids	20 mg/mL	88.10	85.70	-2.7
	10 mg/mL	87.70	87.20	-0.6
	5 mg/mL	88.20	89.20	1.1
Intralipids	20 mg/mL	153.90	157.40	2.2
	10 mg/mL	168.50	166.40	-1.2
	5 mg/mL	168.10	166.30	-1.1
Bilirubin	0.66 mg/mL	14.60	14.90	2.1
	0.2 mg/mL	19.70	19.90	1.2
Bilirubin	0.66 mg/mL	59.20	60.00	1.3
	0.2 mg/mL	85.00	84.30	-0.8

Expected Value:

Random females (n=45), random males (n= 47, COVID-19 positive females (n=19) and COVID-19 positive males (n=31) samples were analyzed using Ansh Labs picoIL-6 ELISA. The expected ranges for Unconjugated Estriol were calculated using 95% non-parametric estimation using Analyse-It® for Microsoft Excel.

Population	n	Median IL-6 Conc. (pg/mL)	IL-6 (pg/mL) 95% CI
Female	45	4.2	0.7 – 69.8
Male	47	4.7	0.8 – 137.6
CoVID-19 Positive Female	19	29.0	4.1 – 1442.4
CoVID-19 Positive Male	31	33.8	6.3 – 1322.8

NOTE: *It is recommended that each laboratory should determine the reference range(s) for its own patient population. The results of this assay should be used in conjunction with other relevant and applicable clinical information.*

REFERENCES

1. Rose-John S: Interleukin-6 signalling in health and disease. F1000Res. 2020 Aug 20;9: F1000 Faculty Rev-1013.
2. Jones SA, Jenkins BJ: Recent insights into targeting the IL-6 cytokine family in inflammatory diseases and cancer. Nat Rev Immunol. 2018; 18:773-89
3. Rose-John S, Winthrop K, Calabrese L: The role of IL-6 in host defence against infections: immunobiology and clinical implications. Nat Rev Rheumatol. 2017 Jul;13(7):399-409
4. Gallo J, Svoboda M, Zapletalova J, et al: Serum IL-6 in combination with synovial IL-6/CRP shows excellent diagnostic power to detect hip and knee prosthetic joint infection. PLoS One. 2018;13: e0199226
5. Song J, Park DW, Moon S, et al: Diagnostic and prognostic value of interleukin-6, pentraxin 3, and procalcitonin levels among sepsis and septic shock patients: a prospective controlled study according to the Sepsis-3 definitions. BMC Infect Dis. 2019; 19:968
6. Galvan-Roman JM, Rodriguez-Garcia SC, Roy-Vallejo, E, et al: IL-6 serum levels predict severity and response to tocilizumab in COVID-19: An observational study. J Allergy Clin Immunol. 2021; 147:72-80
7. Udomsinprasert W, Jittikoon J, Sangroongruangsri S, et al: Circulating levels of interleukin-6 and interleukin-10, but not tumor necrosis factor-alpha, as potential biomarkers of severity and mortality for COVID-19: Systematic review with meta-analysis. J Clin Immunol. 2021; 41:11-22
8. HHS Publication, 5th ed., 2007. Biosafety in Microbiological and Biomedical Laboratories. Available http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5
9. Approved Guideline – Procedures for the Handling and Processing of Blood Specimens, H18-A3. 2004. Clinical and Laboratory Standards Institute.
10. Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures.

Manufactured by:
Ansh Labs
445 Medical Center Blvd.
Webster, TX 77598-4217
U.S.A.