IL-17A (human), ELISA kit

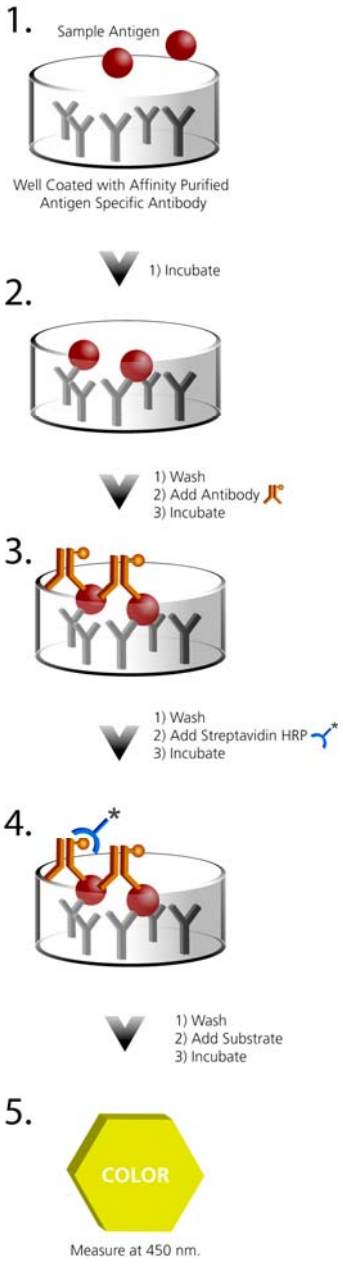
Catalog # ADI-900-177

96 Well Enzyme Immunoassay Kit
For use with serum, plasma, and cell supernatants

Reagents require separate storage conditions.

Check our website for additional protocols, technical notes and FAQs.

Table of Contents



Introduction

The IL-17A (human), ELISA kit is a complete kit for the quantitative determination of IL-17A in plasma, serum and cell supernatants. Please read the complete kit insert before performing this assay.

IL-17, originally identified as CTL-associated antigen 8, is the defining cytokine produced by T_h17 cells, a subset of effector T-helper cells that are known to regulate pathogen clearance, induce autoimmune tissue inflammation, and hematopoiesis[1-4]. Six members of the IL-17 family have been identified and designated IL-17A-F, each possessing a conserved cysteine knot structure. Activation of naïve T cells in the presence of TGF-β and IL-6 initiates differentiation of T_h17 cells. The synergistic actions of IL-6, IL-21 and IL-23 signal through STAT-3 and T_h17-specific transcription factors ROR-γt and ROR-α to amplify the T_h17 response.

IL-17A is produced as a disulfide-linked homodimer that can also form heterodimers with IL-17F. IL-17 acts via ubiquitously expressed IL-17 receptors, inducing expression of pro-inflammatory factors such as IL-1, IL-6, TNF-α, CXCL8/IL-8, and matrix metalloproteinases (MMPs)[5]. Elevated levels of IL-17A and increased presence of T_h17 cells has been associated with airway inflammation, rheumatoid arthritis, intraperitoneal abscesses, inflammatory bowel disease, allograft rejection, psoriasis, multiple sclerosis, and cancer[3,6-8].

Principle

1. Samples and standards are added to wells coated with a polyclonal antibody IL-17A. The plate is then incubated.
2. The plate is washed, leaving only bound IL-17A on the plate. A yellow solution of biotinylated monoclonal antibody to IL-17A is then added. This binds to the IL-17A captured on the plate. The plate is then incubated.
3. The plate is washed to remove excess biotinylated antibody. A blue solution of streptavidin conjugated to horseradish peroxidase is added to each well, binding to the biotinylated monoclonal IL-17A antibody.
4. The plate is washed to remove excess HRP conjugate. TMB Substrate solution is added. An HRP-catalyzed reaction generates a blue color in the solution.
5. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450 nm. The amount of signal is directly proportional to the level of IL-17A in the sample.

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

The standard should be handled with care due to the known and unknown effects of the antigen.

Activity of conjugate is affected by nucleophiles such as azide, cyanide, and hydroxylamine.

Protect substrate from prolonged exposure to light.

Stop solution is caustic. Keep tightly capped.

Materials Supplied

1. **Assay Buffer 13**
   50 mL, Catalog No. 80-1500
   Tris buffered saline containing BSA and detergents

2. **IL-17A Standard**
   lyophilized, Catalog No. 80-2302
   2 vials containing 100pg/vial of recombinant IL-17A

3. **IL-17A Clear Microtiter Plate**
   One plate of 96 wells, Catalog No. 80-2309
   A clear plate of break-apart strips coated with a polyclonal antibody specific for IL-17A

4. **Biotinylated IL-17A Antibody**
   10 mL, Catalog No. 80-2303
   A yellow solution of biotinylated monoclonal antibody to IL-17A

5. **IL-17A Conjugate**
   10 mL, Catalog No. 80-2304
   A blue solution of streptavidin conjugated to horseradish peroxidase

6. **Wash Buffer Concentrate**
   100 mL, Catalog No. 80-1287
   Tris buffered saline containing detergents

7. **TMB Substrate**
   10 mL, Catalog No. 80-0350
   A solution of 3,3’,5,5’ tetramethylbenzidine (TMB) and hydrogen peroxide

8. **Stop Solution 2**
   10 mL, Catalog No. 80-0377
   A 1N solution of hydrochloric acid in water

9. **IL-17A Assay Layout Sheet**
   1 each, Catalog No. 30-0290

10. **Plate Sealer**
    3 each , Catalog No. 30-0012

Reagents require separate storage conditions.

Storage

All components of this kit, except the Standard, are stable at 4°C until the kit's expiration date. The standard must be stored at or below -20°C upon receipt.

Materials Needed but Not Supplied

1. Deionized or distilled water
2. Precision pipets for volumes between 5 µL and 1,000 µL
3. Repeater pipet for dispensing 100 µL
4. Disposable polypropylene beakers
5. Graduated cylinders
6. A microplate shaker
7. Lint-free paper for blotting
8. Microplate reader capable of reading 450 nm
9. Software for extrapolating sample values from optical density readings utilizing a four parameter logistic curve fit.

Reagent Preparation

If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

1. **Wash Buffer**

   Prepare the wash buffer by diluting 50 mL of the supplied Wash Buffer Concentrate with 950 mL of deionized water. This can be stored at room temperature until the kit's expiration date, or for 3 months, whichever comes first.

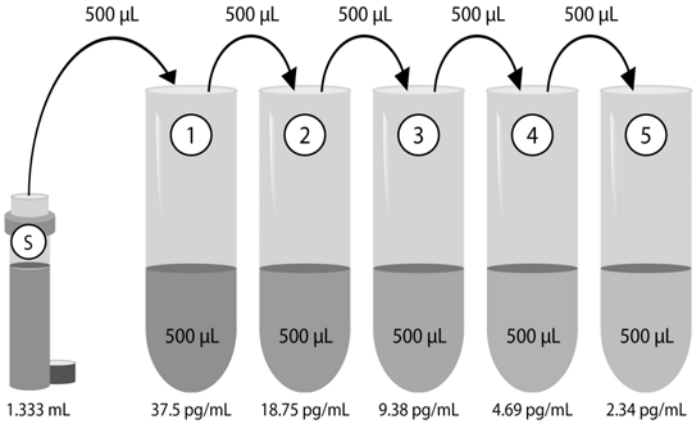
2. **Human IL-17A Standards**

   Allow the 100 pg human IL-17A standard to warm to room temperature. Reconstitute one vial of 100 pg human IL-17A Standard with 1.333 mL standard diluent (Assay Buffer 13 or Tissue Culture Media) for a 75 pg/mL stock vial. Vortex thoroughly, wait 5 minutes and vortex again prior to use.

   Label five disposable polypropylene 12 x 75 mm tubes #2 through #6. Pipet 500 µL standard diluent into each tube. Remove 500 µL from reconstituted stock vial and add to tube #2 and vortex thoroughly. Remove 500 µL from tube #2 and add to tube #3. Vortex thoroughly. Continue this for tubes #3 through #6.

   The standard should be handled with care due to the known and unknown effects of the antigen.

   The concentration of human IL-17A in tubes #1 through #6 will be 75, 37.5, 18.75, 9.38, 4.69, and 2.34 pg/mL respectively (see image below). Standards should be used within 60 minutes of preparation.



Diluted standards should be used within 1 hour of preparation. The concentrations of the standards are labeled above.

Sample Handling

The IL-17A (human), ELISA kit is compatible with human IL-17A samples in culture supernates, plasma and serum. Samples diluted sufficiently into the assay buffer can be read directly from a standard curve. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. A minimum 1:4 dilution is recommended for serum and plasma while cell culture supernates (with 10% serum supplement) may be run neat. These are the minimum recommended dilutions to remove matrix interference in the assay.

Samples must be stored frozen at or below -20° to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.

Samples in the majority of culture media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the culture media instead of Assay Buffer. Culture media may be run neat without matrix interference. Users should only use standard curves generated in media or buffer to calculate concentrations of human IL-17A in the appropriate matrix.

Dilutional Linearity

The minimum required dilution for several common samples was determined by serially diluting samples into the assay buffer and identifying the dilution at which linearity was observed. Conditioned RPMI with 10% fetal bovine serum (FBS) was spiked with recombinant human IL-17A and diluted in the assay buffer. The assay buffer was spiked to the same concentration and used as a control to determine linearity of the culture medium. Multiple samples of Human EDTA plasma and serum were diluted in the assay buffer to produce values within the dynamic range of the assay.

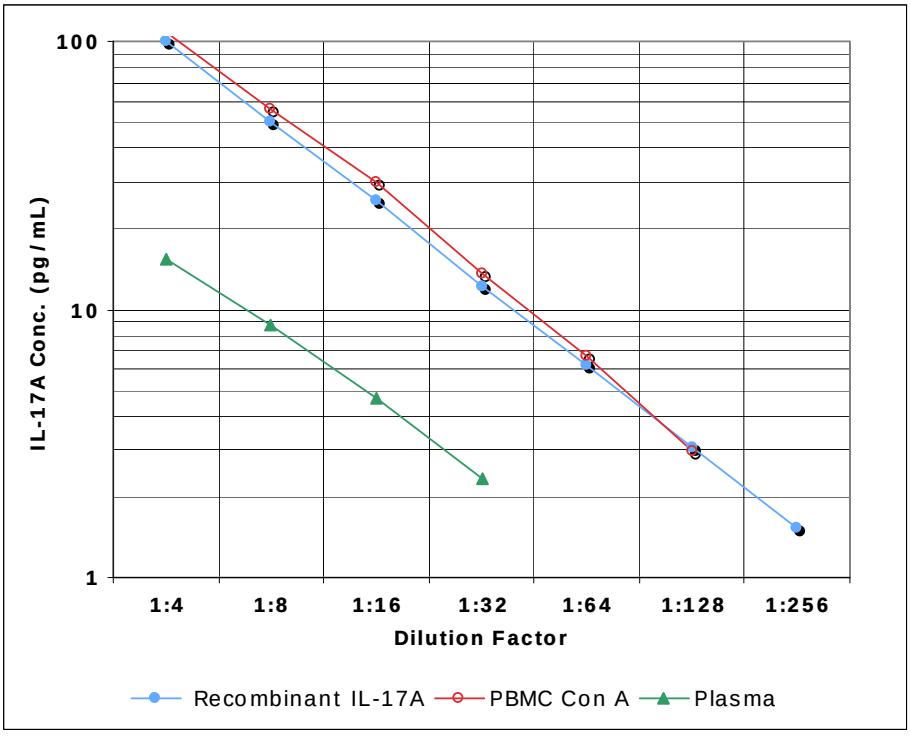
Matrix:	Media
MRD:	Neat
DF	**% Dil. Linearity**
Neat	88.4
1:2	88.5
1:4	95.6
1:8	95.1

Matrix:	Plasma
MRD:	1:4
DF	**% Dil. Linearity**
Neat	37.1
1:2	64.1
1:4	81.8
1:8	93.0

Matrix:	Serum
MRD:	1:4
DF	**% Dil. Linearity**
Neat	27.0
1:2	66.1
1:4	80.7
1:8	91.3

Parallelism

Parallelism experiments were carried out to determine if the recombinant human IL-17A standard accurately determines human IL-17A concentrations in biological matrices. PBMCs (peripheral blood mononuclear cells) stimulated with the mitogen Concanavalin A[9] were used to assess parallelism. Values were obtained using the cell supernatants from treated cultures serially diluted in assay buffer and assessed from a standard curve using four parameter logistic curve fitting. Human EDTA-plasma was similarly diluted in assay buffer and assessed again from a standard curve using four parameter logistic curve fitting. The observed values were plotted against the dilution factors. Parallelism of the curves demonstrates that the antigen binding characteristics are similar enough to allow the accurate determination of native analyte levels in diluted samples.



Plasma Preparation

1. Collect whole blood in appropriate tube.
2. Centrifuge at 1000 x g for 15 minutes at 4˚C.
3. Place supernatant in a clean tube.
4. The supernatant may be aliquoted and stored at or below -20˚C, or used immediately in the assay.
5. Avoid repeated freeze-thaw cycles.

PBMC (Peripheral Blood Mononuclear Cells) Supernate Preparation

1. Incubate PBMCs in RPMI supplemented with 10% FBS, L-glutamine and pen/strep/ampho for 48 hours.
2. Add Concanavalin A at 20 ug/mL. Incubate for 48 hours.
3. Collect cell supernatant.
4. Centrifuge at 600 x g for 10 minutes at room temperature to pellet any cells or cellular debris.
5. Collect supernatant in a clean tube.
6. The supernatant may be aliquoted and stored at or below -20˚C, or used immediately in the assay.
7. Avoid repeated freeze-thaw cycles.

Assay Procedure

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.

Pipet the standards and samples to the bottom of the wells.

Pipet the reagents to the sides of the wells to avoid possible contamination.

Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the foil pouch and seal the ziploc. Store unused wells at 4°C.

1. Pipet 100 µL of standard diluent (Assay Buffer 13 or Culture Media) into the S0 (0 pg/mL standard) wells.
2. Pipet 100 µL of Standards #1 through #6 into the appropriate wells.
3. Pipet 100 µL of the Samples into the appropriate wells.
4. Seal the plate. Incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.
5. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 3 more times for a total of **4 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
6. Pipet 100 µL of yellow biotinylated Antibody into each well, except the Blank.
7. Seal the plate. Incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.
8. Wash as above (Step 5).
9. Add 100 µL of blue Conjugate to each well, except the Blank.
10. Seal the plate. Incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm.
11. Wash as above (Step 5). Pipet 100 µL of Substrate Solution into each well.
12. Seal the plate. Incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm.
13. Pipet 100 µL Stop Solution to each well.
14. Zero the plate reader against the Blank wells, read the optical density at 450 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all the readings.

Calculation of Results

Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

Several options are available for the calculation of the concentration of IL-17A in the samples. We recommend that the data be handled by an immunoassay software package utilizing a four parameter logistic curve fitting program. Such software is often supplied by plate reader manufacturers. If data reduction software is not readily available, the concentration of IL-17A can be calculated as follows.

1. Calculate the average Net OD for each standard and sample by subtracting the average blank OD from the average OD for each standard and sample.

   Average Net OD = Average OD – Average Blanks OD

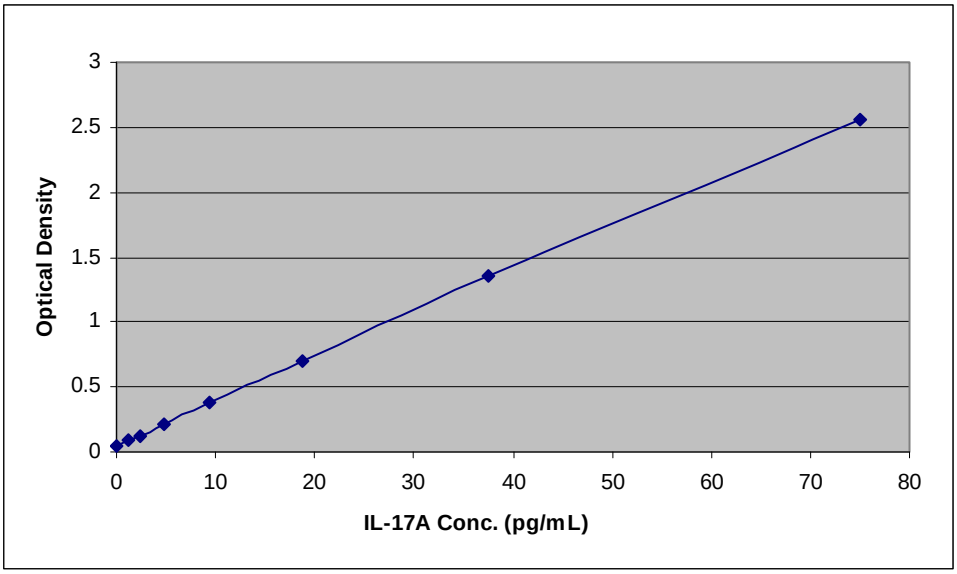
2. Using linear graph paper, plot the average Net OD for each standard versus IL-17A concentration in each standard. Approximate a straight line through the points. The concentration of the unknowns can be determined by interpolation.

Samples with concentrations outside of the standard curve range will need to be reanalyzed using a different dilution.

Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

Sample	Net OD	IL-17A(pg/mL)
S0	0.024	0
S1	2.589	75
S2	1.453	37.5
S3	0.736	18.75
S4	0.363	9.38
S5	0.186	4.69
S6	0.109	2.34



Performance Characteristics

Specificity

The cross reactivity of IL-17C and IL-17F was determined by diluting it in the assay buffer at several concentrations. Cross reactivity was then measured in the assay.

IL-17C		Read from
pg/mL	Mean OD	Std Curve
500000	0.054	0.054
250000	0.051	0.019
50000	0.054	0.049
25000	0.053	0.043
5000	0.073	0.267
2500	0.054	0.054
0	0.055	0.059

IL-17F		Read from
pg/mL	Mean OD	Std Curve
7500	0.068	0
750	0.056	0
75	0.064	0
7.5	0.061	0
0	0.064	0

Sensitivity

The sensitivity or limit of detection of the assay is 0.201 pg/mL. The sensitivity was determined by interpolation at 2 standard deviations above the mean signal at background (0 pg/mL) using data from 6 standard curves.

Precision

Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing IL-17A in a single assay.

pg/mL	%CV
28.08	2.0
7.53	5.4
3.68	10.0

Inter-assay precision was determined by measuring buffer controls of varying IL-17A concentrations in multiple assays over several days.

pg/mL	%CV
26.69	9.1
87.96	9.4
4.12	14.2

References

1. Rouvier, E., et al. (1993) J Immunol. 150, 5445-5456.
2. Steinman, L. (2007) Nat Med. 13, 139-145.
3. Awasthi, A. and Kuchroo, V.K. (2009) Int Immunol. 21, 489-498.
4. Moseley, T.A, et al. (2003) Cytokine Growth Factor Rev. 14, 155-174.
5. Kolls, J.K. and Linden, A. (2004) Immunity 21, 467-476.
6. Tzartos, J.S., et al. (2008) Am J Pathol 172, 146-155.
7. Kebir, H., et al. (2007) Nat Med. 13, 1173-1175.
8. Fujino, S., et al. (2003) Gut 52, 65-70.
9. Lenzrczyk, A., et al. (2000) Clin Exp Immunol 122(1), 41-48.

Notes

Check our website for additional protocols, technical notes and FAQs.

Use of Product

This product contains research chemicals. As such, they should be used and handled only by or under the supervision of technically qualified individuals. This product is not intended for diagnostic or human use.

Warranty

Enzo Life Sciences International, Inc. makes no warranty of any kind, expressed or implied, which extends beyond the description of the product in this brochure, except that the material will meet our specifications at the time of delivery. Enzo Life Sciences International, Inc. makes no guarantee of results and assumes no liability for injuries, damages or penalties resulting from product use, since the conditions of handling and use are beyond our control.

www.enzolifesciences.com

Enabling Discovery in Life Science®

Global Headquarters
Enzo Life Sciences Inc.
10 Executive Blvd
Farmingdale, NY 11735
(p) 1-800-942-0430
(f) 1-631-694-7501
(e) info-usa@enzolifesciences.com

Enzo Life Sciences (ELS) AG
Industriestrasse 17, Postfach
CH-4415 Lause / Switzerland
(p) +41/0 61 926 89 89
(f) +41/0 61 926 89 79
(e) info-ch@enzolifesciences.com

Please visit our website at www.enzolifesciences.com for additional contact information.

Catalog Number: 25-0726