Human IL-17A ELISA Kit

For the quantitative determination of human interleukin-17A (IL-17A) concentrations in serum, plasma, cell culture supernatant, and other biological fluids

Catalogue Number: EL10053

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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TABLE OF CONTENTS

	Page
INTENDED USE	2
INTRODUCTION	2
PRINCIPLE OF THE ASSAY	3
LIMITATION OF THE PROCEDURE	3
REAGENTS PROVIDED	4
MATERIALS REQUIRED BUT NOT SUPPLIED	5
PRECAUTIONS	5
SAMPLE PREPARATION	6
.....Collection, Handling and Storage	6
PREPARATION OF REAGENTS	6
ASSAY PROCEDURE	8
CALCULATION OF RESULTS	9
TYPICAL DATA	9
.....Example one (Calibrator Diluent I)	10
…….Example two (Calibrator Diluent II)	11
PERFORMANCE CHARACTERISTICS	12
.....Intra-assay precision	12
.....Inter-assay precision	12
.....Recovery	12
.....Sensitivity	12
.....Specificity	12
REFERENCES & CITATIONS	13

INTENDED USE

This Human Interleukin-17A ELISA Kit is to be used for the *in vitro* quantitative determination of human interleukin-17A (IL-17A) concentrations in serum, plasma, cell culture supernatant, and other biological fluids. This kit is intended for LABORATORY RESEARCH ONLY and is not to be used for diagnostic or therapeutic procedures.

INTRODUCTION

Interleukin -17A (IL-17A) is a secreted, homodimeric glycoprotein linked by disulfide link with a molecular mass of 35 Kilo Dalton. The cytokine is the proto-type of a newly discovered pro-inflammatory cytokine family which consists of IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25) and IL-17F. All the IL-17s have similar structure with four highly conserved cysteine residues and same 3 dimensional structures, which distinguish this family from other cytokines.

IL-17 is produced by a group of CD4 T help cells termed Th17 cells. In addition to previous discovered Th1 and Th2 cells, the Th17 cells consist in the third group of T help cells. IL-23, a growth and stabilization factor was the first cytokine that was found to involve with the development of Th 17 cells. Later on, other cytokines including TGF-beta, IL-6, IL-21 were also found to contribute to the procedure. The transcription factors implicated in the procedure were identified as STAT3, RORgt, and RORa. IL-17, like interferon-γ, is a potent mediator of delayed-type reaction. IL-17 induces the secretion of many other pro-inflammatory cytokines and chemokines such as IL-6, IL-8, GM-CSF, G-CSF, TGF-beta, TNF-alpha, GRO-alpha, MCP-1, and stimulates the expression of NF-kappaB, mitogen activated protein kinase, and prostaglandings from many cell types. By stimulating cytokine production and recruiting monocytes neutrophils to site of inflammation, the cytokine exerts its effect in response to tissue damage and invasion.

Excessive expression of IL-17 was found to associate with autoimmune diseases and chronic inflammatory diseases such as multiple sclerosis, rheumatoid arthritis, psoriasis, inflammatory bowel diseases, tissue allograft rejection, allergic asthma and infectious diseases at the mucosal tissue.

This IL-17A ELISA is a 3.5-hour solid phase immunoassay readily applicable to measure IL-17A levels in serum, plasma, cell culture supernatant, and other biological fluids in the range of 0 to 1000 pg/mL. It showed no cross reactivity with other cytokines tested. This IL-17A ELISA is expected to be effectively used for further investigations into the relationship between IL-17A and the various conditions mentioned.

PRINCIPLE OF THE ASSAY

This IL-17A enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to IL-17A. Standards or samples are then added to the appropriate microtiter plate wells. A biotin-conjugated antibody preparation specific for IL-17A was added and incubated. IL-17A, if present, will bind and become immobilized by the antibody pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove unbound IL-17A and other components of the sample. Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Avidin is a tetramer containing four identical subunits that each has a high affinity-binding site for biotin. The wells are thoroughly washed to remove all unbound HRP-conjugated Avidin, and a TMB (3,3'5, 5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain IL-17A, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

In order to measure the concentration of IL-17A in the samples this kit includes two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant testing.) According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D) versus IL-17A concentration (pg/mL). The concentration of IL-17A in the samples is then determined by comparing the O.D. of the samples to the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR LABORATORY RESEARCH USE ONLY, NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- As manufacturers we take great care to ensure that our products are suitable for use with all validated sample types, as designated in the product insert. However, it is possible that in some cases, high levels of interfering factors may cause unusual results.
- The kit should not be used beyond the expiration date on the kit label.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

- Soluble receptors or other binding proteins present in biological samples do not necessarily interfere with the measurement of ligands in samples. However, until the factors have been tested, the possibility of interference cannot be excluded.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

	96 tests

1. **IL-17A MICROTITER PLATE** (Part EL53-1) ***96 wells***
   Pre-coated with anti-human IL-17A monoclonal antibody.

2. **BIOTIN CONJUGATE** (Part EL53-2) ***6 mL***
   Anti-human IL-17A monoclonal antibody conjugated to Biotin.

3. **AVIDIN CONJUGATE** (Part EL53-3) ***12 mL***
   Avidin conjugated to horseradish peroxidase.

4. **IL-17A STANDARD** (Part EL53-4) ***2 vials***
   Recombinant human IL-17A (2000pg/vial) in a buffered protein base with preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (EL53-5) ***25 mL***
   Animal serum with buffer and preservative. *For serum/plasma testing.*

6. **CALIBRATOR DILUENT II** (EL53-6) ***25 mL***
   Cell culture medium with calf serum and preservative. *For cell culture supernatant testing.*

7. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

8. **SUBSTRATE A** (Part EL53-7) ***10 mL***
   Buffered solution with H_2O_2

9. **SUBSTRATE B** (Part 30007) ***10 mL***
   Buffered solution with TMB.

10. **STOP SOLUTION** (Part 30008) ***14 mL***
    2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100µL and 50-200µL for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm ± 2nm).
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
   Solid Waste: Autoclave 60 min. at 121°C.
   Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

1. COLLECTION, HANDLING AND STORAGE

a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
b) **Serum**: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.
c) **Plasma:** Blood should be drawn using standard venipuncture techniques with anticoagulant. To ensure optimal recovery and minimal platelet contamination, after collection there must be quick separation of plasma with less than 30 minutes on ice. Centrifuge for 10 minutes (4°C) to remove any particulates.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum, plasma, and cell culture supernatant samples to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

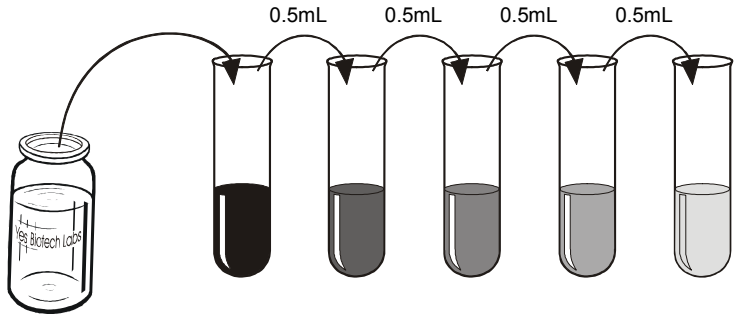
1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table provided for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	2.0	2.0	4.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **IL-17A Standard:**

a) Two vials of Standard are provided in this kit to allow both serum/plasma and cell culture supernatent testing. Reconstitute IL-17A Standard with 2.0 mL of Calibrator Diluent I or Calibrator Diluent II. This reconstitution produces a stock solution of 1000 pg/mL. Allow solution to sit for 5 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The IL-17A standard stock solution can be stored frozen (-20°C) for up to 30 days. Avoid freeze-thaw cycles. Aliquot if repeated use is expected.

b) Use the above stock solution to produce a serial 2-fold dilution series, as described below, within the range of this assay (31.2 to 1000pg/mL) as illustrated. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted IL-17A stock solution will serve as the high standard (**1**000 pg/mL) and the Calibrator Diluent will serve as the zero standard (0 pg/mL).



IL-17A Standard	500 pg/ml	250 pg/ml	125pg/ml	62.5 pg/ml	31.2 pg/ml	1000 pg/ml

ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and IL-17A Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.*

Wells	Contents		**Wells**	Contents	
1A, 1B	Standard 1 - **0 pg/mL**	(S1)	**2A, 2B**	Standard 5 - **250 pg/mL**	(S6)
1C, 1D	Standard 2– **31.25 pg/mL**	(S2)	**2C, 2D**	Standard 6 - **500 pg/mL**	(S7)
1E, 1F	Standard 3- **62.5 pg/mL**	(S3)	**2E, 2F**	Standard 7- **1000 pg/mL**	(S7)
1G, 1H	Standard 4– **125 pg/mL**	(S4)	**2G, 2H**	Sample	

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S1	S5	2	6	10	14	18	22	26	30	34	38
B	S1	S5	2	6	10	14	18	22	26	30	34	38
C	S2	S6	3	7	11	15	19	23	27	31	35	39
D	S2	S6	3	7	11	15	19	23	27	31	35	39
E	S3	S7	4	8	12	16	20	24	28	32	36	40
F	S3	S7	4	8	12	16	20	24	28	32	36	40
G	S4	1	5	9	13	17	21	25	29	33	37	41
H	S4	1	5	9	13	17	21	25	29	33	37	41

2. Add 100µL of Standard or Sample to the appropriate well of the antibody pre-coated Microtiter Plate and incubate 1 hour at room temperature.

3. Without discarding the standards and samples, add 50µL Anti-IL-17A Biotin conjugate to each wells. Mix well. Cover and incubate for 1 hour at room temperature.

4. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

5. Dispense 100µl of Avidin Conjugate to each well Mix well. Cover and incubate for 1 hour at room temperature.

6. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).

7. Repeat wash procedure as described in Step 4.

8. Add 100µL Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.

9. Add 100µL Stop Solution to each well. Mix well.

10. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader set within 30 minutes.

CALCULATION OF RESULTS

The standard curve is used to determine the amount of IL-17A in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding IL-17A concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 pg/mL)) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of IL-17A in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding IL-17A concentration. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

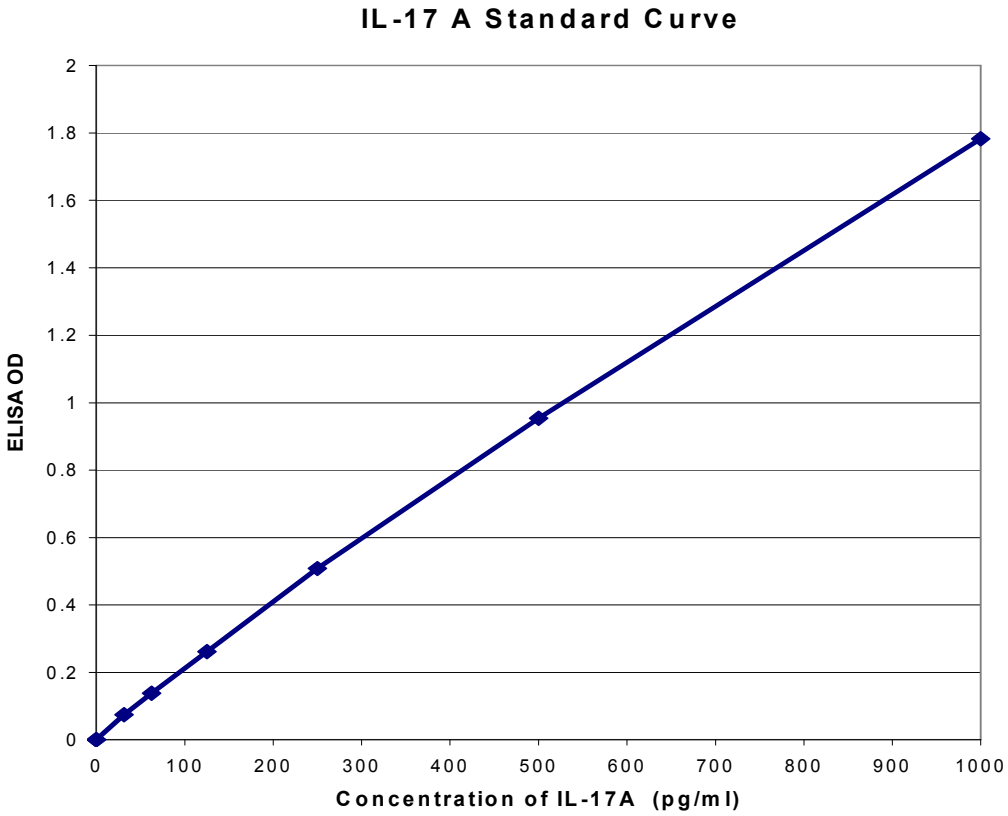
TYPICAL DATA

Results of a typical standard run of an IL-17A ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of *illustration only*, and should not be used to calculate unknowns. Each user should obtain their own standard curve.

EXAMPLE ONE

The following data was obtained for a standard curve using Calibrator Diluent I.

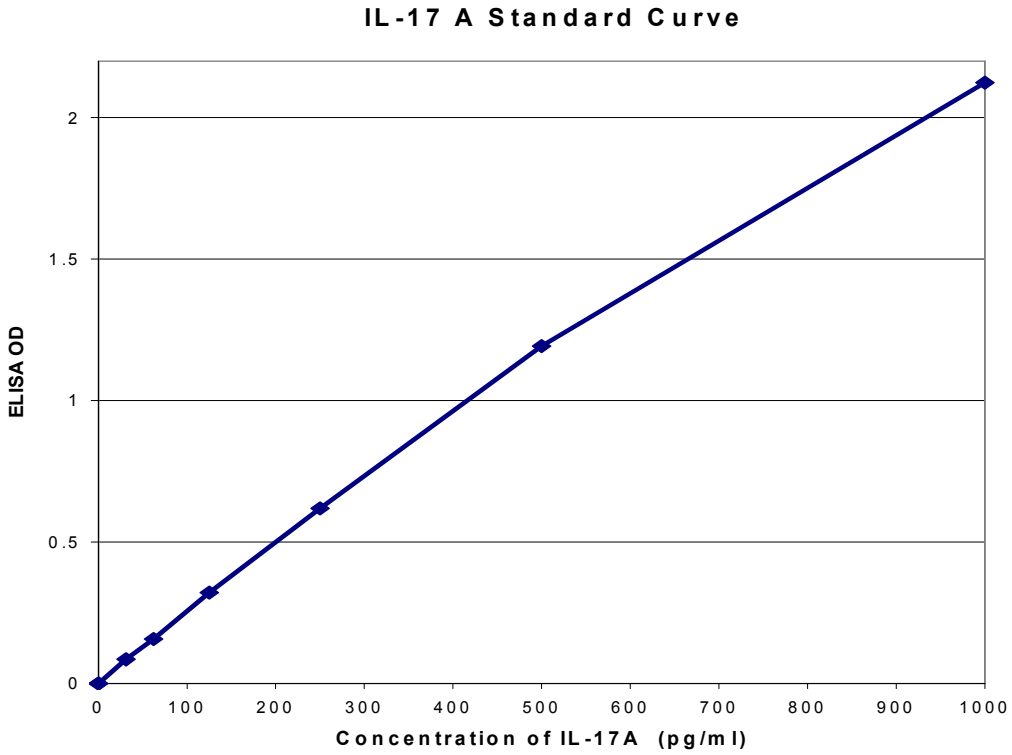
Standard (pg/mL)	Mean O.D. (450 nm)	CV	Zero Standard Subtracted (Std.) - (S1)
0	0.056	5.05	0.000
31.2	0.130	4.35	0.074
62.5	0.194	3.27	0.138
125	0.317	3.12	0.261
250	0.564	0.05	0.508
500	1.010	2.10	0.954
1000	1.839	0.69	1.783



EXAMPLE TWO

The following data was obtained for a standard curve using Calibrator Diluent II.

Standard (pg/mL)	Mean O.D. (450 nm)	CV	Zero Standard Subtracted (Std.) - (S1)
0	0.052	11.3	0.000
31.2	0.137	7.23	0.085
62.5	0.209	6.77	0.157
125	0.373	0.38	0.321
250	0.671	1.79	0.619
500	1.245	1.70	1.193
1000	2.176	0.23	2.124



PERFORMANCE CHARACTERISTICS

1. **INTRA-ASSAY PRECISION**
   To determine within-run precision, three different samples of known concentration were assayed by replicates of twenty in 1 assay.

Sample	1	2	3
N	18	18	18
Mean (pg/ml)	106	218	506
Coefficient of Variation (%)	4.36	3.80	2.24

2. **INTER-ASSAY PRECISION**
   To determine between-run precision, three different samples of known concentration were assayed by replicates on 6 different assays.

Sample	1	2	3
N	6	6	6
Mean (pg/mL)	123	243	573
Coefficient of Variation (%)	9.7	6.5	7.1

3. **RECOVERY**
   The recovery of IL-17A spiked to different levels throughout the range of the assay was evaluated.

Sample Type	Average Recovery %	Range %
Cell culture media	91.1	84.8 -101.2
Serum	106.3	103-111
Plasma	93.34	88.2 – 95.0

4. **SENSITIVITY**
   The minimum detectable dose of IL-17A was determined by adding two standard deviations to the mean optical density value of 20 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose of human IL-17A calculated from calibrate I diluted standard curve was <5 pg/mL.

5. **SPECIFICITY**
   This sandwich ELISA recognises both natural and recombinant human IL-17A. This kit has been tested and exhibited no significant cross-reactivity with following human cytokines and growth factors: IL-1α, IL-1β, IL-2, IL-4, IL-5, Il-6, IL-7, IL-8, IL-10, IL-11, Il-12, Il-13Ra2, FGF basic, GM-CSF, IFN-γ, M-CSF, MCP-1(MCAF), MCP3, EGF, TNF- α, TNF-β.

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