Human IL-22 ELISA Kit

For the quantitative determination of human interleukin 22 (IL-22) concentrations in human serum, cell culture supernatant, and other biological fluids.

Catalogue Number: EL10055

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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S7.5 (08/13-01) IL-22

TABLE OF CONTENTS

	Page

INTENDED USE	2
INTRODUCTION	2
PRINCIPLE OF THE ASSAY	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
PERFORMANCE CHARACTERISTICS	12
.............Intra-assay precision	12
.............Inter-assay precision	12
.............Recovery	12
.............Sensitivity	12
REFERENCES	13

INTENDED USE

This Human IL-22 ELISA kit is to be used for the *in vitro* quantitative determination of human interleukin 22 (IL-22) concentrations in serum, cell culture supernatant, and other biological fluids. This kit is intended for LABORATORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

INTRODUCTION

Interleukin 22 (IL-22) was identified in 2000 as an IL-10 related cytokine that signals through class II cytokine receptor proteins (1). IL-22 signaling is different from IL-10 since some cell lines respond to IL-22 by activating STAT (Signal Transducer and Activator of Transcription) proteins, but are unresponsive to IL-10 (1). The counterpart of human IL-22 in mouse is called IL-TIF (3). Il-22 functions through IL-22 receptor (IL-22R), and a common IL-10R2 receptor that is shared by other members of the IL-10 family (2). A natural antagonist of IL-22, IL-22 binding protein, was found to down-regulate IL-22 function (4).

IL-22 is produced by dendritic cells, T-cells and natural killer cells during bacterial infection, auto-immunity and tissue inflammation (6, 7). IL-22 acts upon innate immunity cells through its receptors expressed exclusively on these cells. In CD4+ T helper cells, IL-22 expression has been found to be associated with Th17 and Th1. Recently, an IL-22 expressing T helper cell subset (Th22) was characterized (10) which is distinct from other T cells by coexpression of the chemokine receptor CCR6 and the skin-homing receptors CCR4 and CCR10. IL-22 expression is elevated in psoriatic skin inflammation (5, 8, 11, 12), atopic dermatitis (13), inflammatory bowel disease (14). In cutaneous T-cell lymphoma, IL-22 dominates the tumor microenvironment and STAT3 phosphorylation was observed (17). IL-22 was also found to promote murine hepatocyte survival (16) and ameliorate intestinal inflammation in mouse ulcerative colitis model (9).

PRINCIPLE OF THE ASSAY

This human IL-22 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 for human IL-22. Standards or samples are then added to the appropriate microtiter plate wells and incubated. Human IL-22, if present, will bind and become immobilized by the antibody pre-coated on the wells. Then, a preparation of biotin conjugated detection antibody for human IL-22 is added to each well and incubated. The Biotin conjugated antibody will bind to human IL-22 during incubation. The microtiter plate wells are thoroughly washed to remove unbound components in the samples and biotin conjugate preparation. Avidin has a very high affinity to biotin. In order to quantify the amount of human IL-22 present in the sample, a preparation of horseradish peroxidase (HRP)-conjugated Avidin is added to each well. HRP will be linked to the IL-22 detection antibody through high affinity binding of Biotin and Avidin, and connect indirectly with human IL-22 immobilized on the well. After incubation, the wells are thoroughly washed to remove all unbound HRP-conjugated antibodies 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 human IL-22 will exhibit a change in colour and the extent of colour change is proportional to the amount of human IL-22 in standards/samples. 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 human IL-22 in the samples, this kit contains two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant/urine 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-22 concentration (pg/mL). The concentration of IL-22 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

This IL-22 ELISA is a 3.5-hour solid-phase immunoassay readily applicable to measure IL-22 levels in serum, plasma, cell culture supernatant, and other biological fluids in the range of 0 to 600pg/mL.

REAGENTS PROVIDED

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

96 tests

1. **HUMAN IL-22 MICROTITER PLATE** (Part EL55-1)______________ ***96 wells***
   Microtiter plate pre-coated with anti-human IL-22 monoclonal antibody.

2. **BIOTIN CONJUGATE** (Part EL55-2)______________ ***6 mL***
   Anti-human IL-22 monoclonal antibody conjugated to Biotin.

3. **AVIDIN HRP CONJUGATE** (Part EL55-3)______________ ***12 mL***
   Avidin conjugated to horseradish peroxidase

4. **HUMAN IL-22 STANDARD** (Part EL55-4)______________ ***2 vials***
   Recombinant human IL-22 (1200pg/vial) in a buffered protein base with preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (Part EL55-5)______________ ***25 mL***
   Animal serum with preservative. *For serum testing*.

6. **CALIBRATOR DILUENT II** (Part EL55-6)______________ ***25 mL***
   Cell culture medium with calf serum and preservative. *For cell culture supernatant/urine testing.*

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

8. **SUBSTRATE A** (Part EL55-7) ______________ ***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 50-2200μL required 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 Wastes: Autoclave for 60 minutes at 121°C.

   Liquid Wastes: 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 viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate contains 20% acetone: Keep this reagent away from sources of heat and 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.

- Avoid hemolytic, lipidic or turbid samples.
- Serum and cell culture supernatant that are to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must be 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.

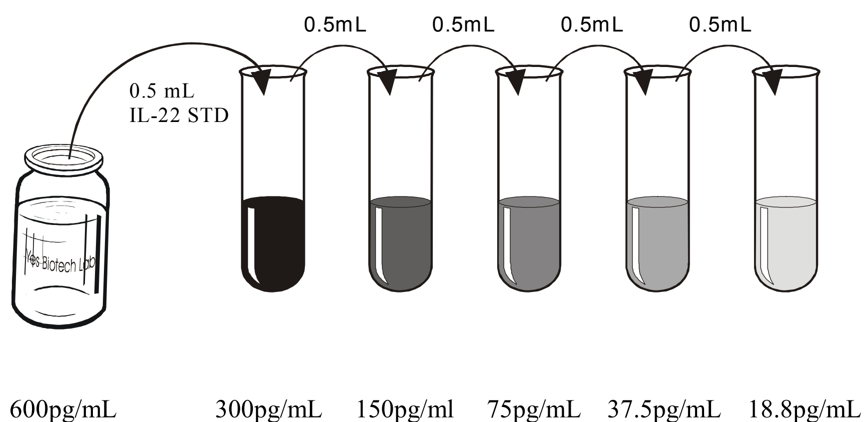
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the following table for the correct amount of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
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. **Human IL-22 Standard**:

a) Two vials of Standards are provided in this kit to allow both serum and cell culture supernatant testing. Reconstitute the human IL-22 Standard with either 2.0mL of Calibrator Diluent I (for serum testing) or Calibrator Diluent II (for cell culture supernatant testing). This reconstitution produces a stock solution of 600pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. Avoid freeze-thaw cycles: aliquot if repeated use is expected.

b) Use the above stock solution to produce a 2-fold dilution series within the range of this assay (18.8pg/mL to 600pg/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-22 Standard will serve as the high standard (600pg/mL) and the Calibrator Diluent will serve as the zero-standard (0pg/mL).



ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and human IL-22 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 – **150 pg/mL**	(S5)
1C, 1D	Standard 2 –**18.8 pg/mL**	(S2)	**2C, 2D**	Standard 6 – **300 pg/mL**	(S6)
1E, 1F	Standard 3 – **37.5 pg/mL**	(S3)	**2E, 2F**	Standard 7 - **600 pg/mL**	(S7)
1G, 1H	Standard 4 – **75 pg/mL**	(S4)	**2G,2H** **3A-12H**	**IL-22 samples**	

	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. Cover and incubate for 1 hour at room temperature.

3. Without discarding the standards and samples, add 50μL Biotin conjugated anti-human IL-22 antibody 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 350μL/well/wash (range: 350-400μL). 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 HRP conjugate to each well. Cover and incubate for 30 minutes at room temperature.

6. Repeat wash procedure as described in Step 4.

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

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

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

CALCULATION RESULT

The standard curve is used to determine the amount of human IL-22 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-22 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-22 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-22 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.

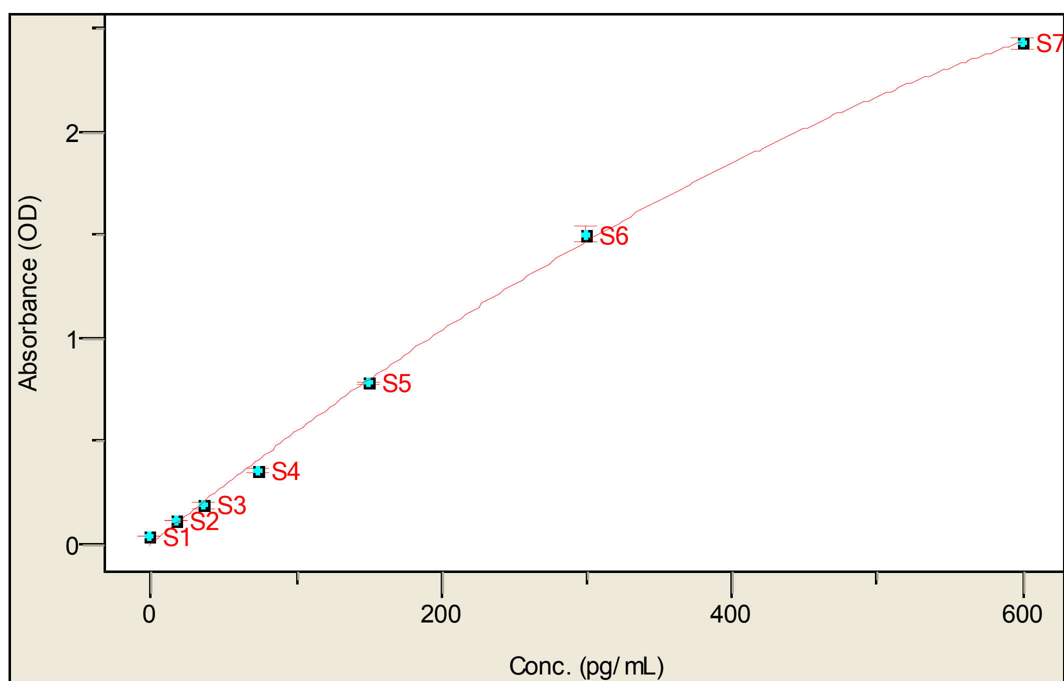
TYPICAL DATA

Results of a typical standard run of a human IL-22 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 user results.

EXAMPLE ONE

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

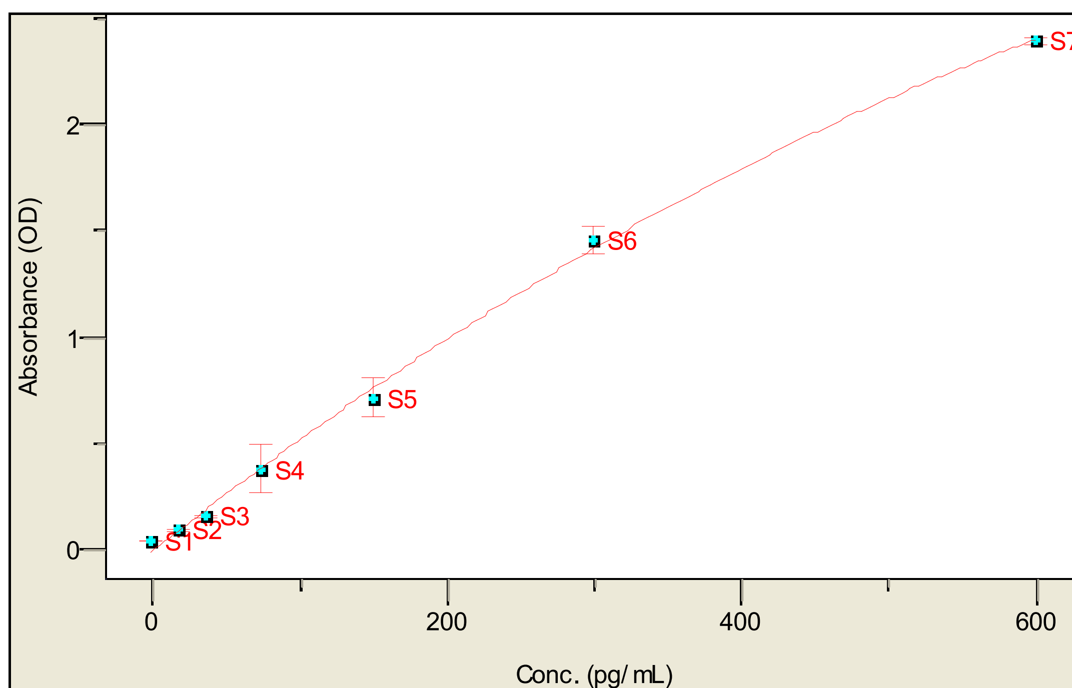
Standard (pg/mL)	Mean O.D. (450 nm)	%CV	Zero Standard Subtracted (Std.)-(S1)
0	0.0425	1.66	0
18.75	0.1200	1.01	0.0775
37.5	0.1900	6.70	0.1475
75	0.3605	4.12	0.3180
150	0.7840	1.08	0.7415
300	1.5070	2.35	1.4645
600	2.4330	1.05	2.3905



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	0.0435	1.63	0
18.8	0.0995	3.55	0.056
37.5	0.1620	5.24	0.1185
75	0.3865	2.95	0.343
150	0.7220	1.23	0.6785
300	1.4310	4.36	1.3875
600	2.3970	5.31	2.3535



PERFORMANCE CHARACTERISTICS

1. **INTRA-ASSAY PRECISION**
   To determine within-run precision, three different samples of known concentration were assayed by using 16 replicates in 1 assay.

Sample	**1**	**2**	**3**
N	16	16	16
Mean (pg/ml)	38.2	183.3	459.4
Coefficient of Variation (%)	6.64	7.25	5.89

2. **INTER-ASSAY PRECISION**
   To determine between-run precision, three different samples of known concentration were assayed by using replicates on 8 different assays.

	Calibrator Diluent II assay		
Sample	**1**	**2**	**3**
N	8	8	8
Mean (pg/mL)	40.6	196.6	507.1
Coefficient of Variation (%)	9.2	10.6	10.6

3. **RECOVERY**
   The recovery of human IL-22 at three different levels in cell culture medium and human serum was evaluated with human IL-22 spiked samples.

Sample Type	Average Recovery %	Range %
Cell culture media	94.1	84.8 -103.5
Human Serum	94.6	88.0-99.2

4. **SENSITIVITY**
   The minimum detectable dose of human IL-22 was determined by adding two standard deviations to the mean optical density value of 16 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose of human IL-22 calculated from calibrate I diluted standard curve was <9.2 pg/mL.

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