

Human IL-22 ELISA Kit

Instructions for use

Catalogue numbers: 1x96 tests: 650.140.096
 2x96 tests: 650.140.192

For research use only

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Human IL-22 ELISA KIT

1. Intended use

This Human IL-22 ELISA is an enzyme-linked immunosorbent assay for quantitative detection of human IL-22 levels in cell culture supernatants, human serum and plasma.

This kit has been configured for research use only.

2. Introduction

2.1. Summary

IL-22/TIF (IL-10-related T cell-derived inducible factor) is a new cytokine originally identified as a gene induced by IL-9 in murine T lymphocytes, and showing 22% amino acid identity with IL-10. In the mouse, the IL-22 gene is located on chromosome 10, in the same region as the IFN gamma gene. Although it is a single copy gene in BALB/c and DBA/2 mice, the IL-22 gene is duplicated in other strains such as C57Bl/6, FVB and 129. The two copies, which show 98% nucleotide identity in the coding region, were named IL-TIF alpha and IL-TIF beta with the IL-TIF beta gene being either differentially regulated, or not expressed at all.

IL-22 is produced by activated Th1 and NK cells acting primarily on epithelial cells and is involved in inflammatory responses. Neither resting nor activated immune cells express IL-22 receptor, and IL-22 does not have any effects on these cells in vitro and in vivo. In contrast, cells of the skin and the digestive and respiratory systems represent putative targets of this cytokine. Thus IL-22 does not serve the communication between immune cells but is a T cell mediator that directly promotes the innate, nonspecific immunity of tissues. IL-22 serves as a protective molecule to counteract the destructive nature of the immune response to limit tissue damage.

Interleukin-22 (IL-22) is a cytokine that regulates the production of acute phase proteins of the immunological response. On binding to its cognate receptor (IL-22R1), which is associated to the interleukin-10 receptor 2 (IL-10R2), IL-22 promotes activation of signal transducer and activator of transcription (STAT) pathway and several other cellular responses. A soluble receptor termed interleukin-22 binding protein (IL-22BP) is also able to bind to IL-22 as a natural protein antagonist, and probably provides systemic regulation of IL-22 activity.

IL-22, in contrast to its relative IFN-gamma, regulates the expression of only a few genes in keratinocytes. This is due to varied signal transduction. The IL-22 effects are transcriptional and either independent of protein synthesis and secretion, or mediated by a secreted protein. Inflammatory conditions, but not keratinocyte differentiation, amplify the IL-22 effects. IL-22 application in mice enhances cutaneous S100A9 and MMP1 expression.

Psoriatic patients show strongly elevated IL-22 plasma levels, which correlated with the disease severity. IL-22 plays a protective role in T cell-mediated hepatitis induced by Concanavalin A (Con A), acting as a survival factor for hepatocytes.

IL-22 is present in high quantities in the blood of Crohn's disease patients in contrast to IFN-gamma and IL-17

2.2. Principle of the method

An anti-IL-22 monoclonal coating antibody is adsorbed onto microwells. IL-22 present in the sample or standard binds to antibodies adsorbed to the microwells. In the next incubation Biotin-conjugated monoclonal anti-IL-22 antibody binds to IL-22 captured by the first antibody.

Following incubation unbound Biotin conjugated anti-IL-22 is removed during a wash step and substrate solution reactive with Biotin is added to the wells.

A coloured product is formed in proportion to the amount of IL-22 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from seven IL-22 standard dilutions and an IL-22 sample concentration determined

3. Reagents provided and reconstitution

Reagents (Store@2-8°C)	Quantity 1x96 well kit Cat no. 650.140.096	Quantity 2x96 well kit Cat no. 650.140.192	Reconstitution
96-wells precoated microtiter plate	1	2	Ready-to-use
Plate covers	2	4	
Standard 4 ng/ml	2 vials	4 vials	Reconstitute with the volume of distilled water indicated on the vial.
Biotin anti-human IL-22	1 vial	2 vials	(0.14ml) Make a 1/100 dilution in Assay Buffer
Assay Buffer Concentrate	1 vial	2 vials	(5ml) 20X concentrate. Dilute in distilled water
Streptavidin-HRP	1 vial	2 vials	(0.15ml) Make a 1/200 dilution in Assay Buffer
Wash Buffer Concentrate	1 vial	2 vials	(50ml) 20X concentrate. Dilute in distilled water
Sample Diluent	1 vial	2 vials	(12 ml) Ready-to-use
Substrate Solution	1 vial	2 vials	(15 ml) Ready- to- use
Stop Solution	1 vial	2 vials	(15 ml) Ready-to-use
Blue Dye	1 vial	2 vials	(0.4 ml) Make a 1/250 dilution in the appropriate diluent
Red Dye	1 vial	2 vials	(0.4 ml) Make a 1/250 dilution in the appropriate diluent
Green Dye	1 vial	2 vials	(0.4 ml) Make a 1/100 dilution in the Assay Buffer

4. Materials required but not provided

- Microtiter plate reader fitted with appropriate filters (450nm required with optional 630nm reference filter)
- Microplate washer or wash bottle
- 10, 50, 100, 200 and 1,000µl adjustable single channel micropipettes with disposable tips
- 50-300µl multi-channel micropipette with disposable tips
- Multichannel micropipette reagent reservoirs
- Distilled water
- Vortex mixer
- Orbital Shaker (rotator)
- Miscellaneous laboratory plastic and/or glass, if possible sterile

5. Storage Instructions

Store kit reagents between 2°C and 8°C except controls. Store lyophilized controls at -20°C. Immediately after use reagents should be returned to cold storage (2°C to 8°C), controls to -20°C, respectively. Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

6. Specimen collection, processing & storage

Cell culture supernatants, plasma, and human serum will be suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be stored frozen at -20°C, unless they will be assayed the day of collection. Excessive freeze-thaw cycles should be avoided. Prior to assay, frozen sera should be brought to room temperature slowly and mixed gently. Do not thaw samples in a 37°C water bath. Do not vortex or sharply agitate samples.

7. Safety & precautions for use

- Handling of reagents, serum or plasma specimens should be in accordance with local safety procedures , e.g.CDC/NIH Health manual : " Biosafety in Microbiological and Biomedical Laboratories" 1984
- Laboratory gloves should be worn at all times
- Avoid any skin contact with H₂SO₄ and TMB. In case of contact, wash thoroughly with water
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used
- Do not pipette by mouth
- When not in use, kit components should be stored refrigerated or frozen as indicated on vials or bottles labels
- All reagents should be warmed to room temperature before use. Lyophilized standards should be discarded after use
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration
- Cover or cap all reagents when not in use
- Do not mix or interchange reagents between different lots
- Do not use reagents beyond the expiration date of the kit
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross contamination, for the dispensing of H₂SO₄ and substrate solution, avoid pipettes with metal parts
- Use a clean plastic container to prepare the washing solution
- Thoroughly mix the reagents and samples before use by agitation or swirling
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells
- The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent colour development. Warning TMB is toxic avoid direct contact with hands. Dispose off properly
- If a dark blue colour develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbance's within 1 hour after completion of the assay
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells
- Follow incubation times described in the assay procedure
- Dispense the TMB solution within 15 min of the washing of the microtitre plate

8. Assay Preparation

Bring all reagents to room temperature before use

8.1. Assay Design

Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running zeros and standards. Each sample, standard and zero should be tested **in duplicate**. Remove sufficient Microwell Strips for testing from the aluminium pouch immediately prior to use. Return any wells not required for this assay with desiccant to the pouch. Seal tightly and return to 2-8°C storage.

Example plate layout (example shown for a 6 point standard curve)

	Standards		Sample Wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	2000	2000										
B	1000	1000										
C	500	500										
D	250	250										
E	125	125										
F	62.5	62.5										
G	31.3	31.3										
H	Zero	Zero										

All remaining empty wells can be used to test samples in duplicate

8.2. Preparation of Wash Buffer

If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved.

Pour entire contents (50 ml) of the **Wash Buffer Concentrate** into a clean 1,000 ml graduated cylinder. Bring final volume to 1,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that the Wash Buffer is stable for 30 days.

8.3. Preparation of Assay Buffer

Mix the contents of the bottle well. Add contents of **Assay Buffer Concentrate** (5.0 ml) to 95 ml distilled or deionized water and mix gently to avoid foaming. Store at 2° to 8°C. Please note that the Assay Buffer is stable for 30 days.

8.4. Preparation of Standard

Standard vials must be reconstituted with the volume of distilled water shown on the vial immediately prior to use. This reconstitution gives a stock solution of 4000pg/ml of IL-22. **Mix the reconstituted standard gently by inversion only.** Serial dilutions of the standard are made directly in the assay plate to provide the concentration range from 2000 to 31.3pg/ml. A fresh standard curve should be produced for each new assay.

- Add 100µl of Sample Diluent to the standard wells A1 and A2 to G1 and G2
- Immediately after reconstitution add 100µl of the reconstituted standard to wells A1 and A2, which provides the highest concentration standard at 2000pg/ml
- Transfer 100µl from wells A1 and A2 to B1 and B2. Mix the well contents by repeated aspirations and ejections taking care not to scratch the inner surface of the wells
- Continue this 1:1 dilution using 100µl from wells B1 and B2 through to wells G1 and G2 providing a serial diluted standard curve ranging from 2000 to 31.3pg/ml
- Discard 100µl from the final wells of the standard curve (G1 and G2)

Alternatively these dilutions can be performed in separate clean tubes and immediately transferred directly into the relevant wells.

8.5. Preparation of Biotin-Conjugate

Make a 1:100 dilution of the concentrated **Biotin Conjugate** with **Assay Buffer** in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (ml)
1 - 6	0.06	5.94
1 - 12	0.12	11.88

8.6. Preparation of Streptavidin-HRP

Make a 1:200 dilution of the concentrated **Streptavidin HRP** with **Assay Buffer** in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (ml)	Assay Buffer (ml)
1 - 6	0.03	5.97
1 - 12	0.06	11.94

8.7. Addition of colour-giving reagents: Blue-Dye, Green-Dye.

In order to help our customers to avoid any mistakes in pipetting, Color dyes use helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colours to each step of the ELISA procedure. This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.

Alternatively, the dye solutions from the stocks provided (**Blue-Dye, Green-Dye**) can be added to the reagents according to the following guidelines:

1. Diluent: Before sample dilution add the **Blue-Dye** at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol. After addition of **Blue-Dye**, proceed according to the instruction booklet.

5 ml Assay Buffer	20 µl Blue-Dye
12 ml Assay Buffer	48 µl Blue-Dye
50 ml Assay Buffer	200 µl Blue-Dye

2. Biotin-Conjugate: Before dilution of the concentrated conjugate, add the **Green-Dye** at a dilution of 1:100 (see table below) to the Assay Buffer used for the final conjugate dilution. Proceed after addition of **Green-Dye** according to the instruction booklet, preparation of Biotin-conjugate.

6 ml Assay Buffer	60 µl Green-Dye
12 ml Assay Buffer	120 µl Green-Dye

3. Streptavidin HRP: Before dilution of the concentrated Streptavidin HRP, add the **Red-Dye** at a dilution of 1:250 (see table below) to the Assay Buffer used for the Streptavidin dilution. Proceed after addition of **Red-Dye** according to the instruction booklet, preparation of Streptavidin HRP.

6 ml Assay Buffer	24 µl Red-Dye
12 ml Assay Buffer	48 µl Red-Dye

9. Method

We strongly recommend that every vial is mixed thoroughly without foaming prior to use except the standard vial which must be mixed gently by inversion only.

Prepare all reagents as shown in section 8.

Assay Step		Details
1.	Wash	Remove the pre-coated plate from the sealed pouch, removed any un-needed strips and wash the plate as follows: a) Dispense 0.4ml of 1x washing solution into each well b) Aspirate the contents of each well c) Repeat step a and b
2.	Addition	Prepare Standard curve as shown in section 7.4
3.	Addition	Add 100µl of Sample Diluent in duplicate to the blank wells
4.	Addition	Add 50µl of Sample Diluent to the sample wells
5.	Addition	Add 50µl of each sample in duplicate to the designated wells
6.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for 2 hours on a rotator set at 400rpm if available
7.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well b) Dispense 0.3 ml of 1x washing solution into each well c) Aspirate the contents of each well d) Repeat step b and c another two times
8.	Addition	Add 50µl of diluted biotinylated Conjugate to all wells including blanks
9.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for 1 hours on a rotator set at 400rpm if available
10.	Wash	Repeat wash step 7.
11.	Addition	Add 100µl of Streptavidin-HRP solution into all wells
12.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for 1 hour on a rotator set at 400rpm if available
13.	Wash	Repeat wash step 7.
14.	Addition	Add 100µl of ready-to-use TMB Substrate Solution into all wells
15.	Incubation	Incubate for 10 minutes* at room temperature on a rotar set at 200rpm if available. Avoid direct exposure to light by wrapping the plate in aluminium foil
16.	Addition	Add 100µl of Stop Reagent into all wells
<p>Read the absorbance value of each well (immediately after step 16.) on a spectrophotometer using 450 nm as the primary wavelength and optionally 630 nm as the reference wave length (610 nm to 650 nm is acceptable).</p>		

**Incubation time of the substrate solution is usually determined by the ELISA reader performance. Many ELISA readers only record absorbance up to 2.0 O.D. Therefore the colour development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range*

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

10. Data Analysis

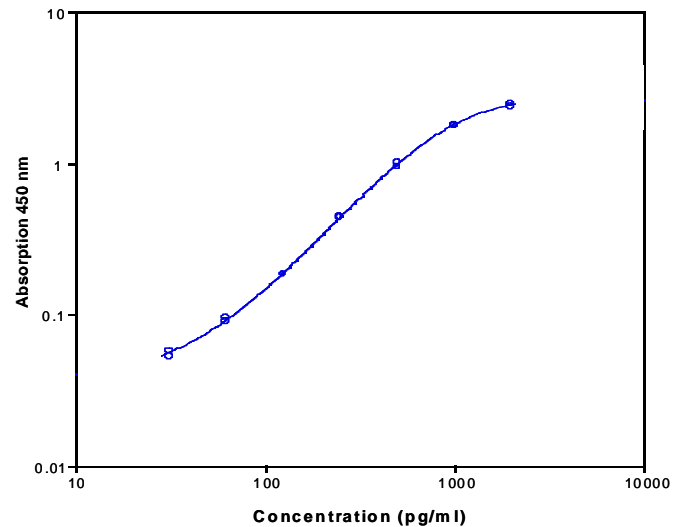
Calculate the average absorbance values for each set of duplicate standards and samples. Ideally duplicates should be within 20% of the mean.

Generate a best fit standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding murine IL-22 standard concentration on the horizontal axis.

The amount of IL-22 in each sample is determined by extrapolating OD values against IL-22 standard concentrations using the standard curve.

Example of IL-22 standard curve

Standard	IL-22 Conc pg/ml	OD (450nm) Mean	CV (%)
1	2000	2.412	3.7
2	1000	1.792	1.4
3	500	0.980	5.2
4	250	0.439	1.2
5	125	0.185	0.3
6	62.5	0.093	4.3
7	31.3	0.055	4.8
Zero	0	0.018	



Note; curve shown above should not be used to determine results. Every laboratory must produce a standard curve for each set of microwell strips assayed.

For samples which have been diluted 1:2 according to the protocol, the calculated concentration should be multiplied by the dilution factor (x2)

11. Assay limitations

Do not extrapolate the standard curve beyond the maximum standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples above the maximum standard concentration must be diluted with Standard diluent or with your own sample buffer to produce an OD value within the range of the standard curve. Following analysis of such samples always multiply results by the appropriate dilution factor to produce actual final concentration.

The influence of various drugs on end results has not been investigated. Bacterial or fungal contamination and laboratory cross-contamination may also cause irregular results.

Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Washing Buffer, fill with Washing Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

As with most biological assays conditions may vary from assay to assay therefore **a fresh standard curve must be prepared and run for every assay**

12. Performance Characteristics

12.1. Sensitivity

The limit of detection of Human IL-22 defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be **5.0pg/ml** (mean of 6 independent assays).

12.2. Specificity

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human IL-22 positive serum.

There was no cross reactivity detected.

12.3. Precision

Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of IL-22. Two standard curves were run on each plate. **The overall intra-assay coefficient of variation has been calculated to be 6.7%.**

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by three technicians. Each assay was carried out with 6 replicates of 8 samples containing different concentrations of IL-22. Two standard curves were run on each plate. **The overall inter-assay coefficient of variation has been calculated to be 4.5%.**

12.4. Dilution Parallelism

Serum, plasma and cell culture supernatant samples with different levels of human IL-22 were analysed at serial 2 fold dilutions with 4 replicates each.

For recovery data see Table below

Sample Matrix	Recovery of Exp. Val. (%)
Serum	107
Plasma (EDTA)	105
Plasma (citrate)	93
Plasma (heparin)	76
Cell culture supernatant	83

12.5. Spike Recovery

The spike recovery was evaluated by spiking 3 levels of human IL-22 into serum, plasma (EDTA, citrate, heparin) and cell culture supernatant samples. Recoveries were determined in 3 independent experiments with 4 replicates each.

The amount of endogenous human IL-22 in unspiked serum, plasma and cell culture supernatant samples was subtracted from the spike values.

For the overall mean recovery see Table below

Sample matrix	Mean recovery (%)
Serum	92
Plasma (EDTA)	60
Plasma (citrate)	72
Plasma (heparin)	124
Cell culture supernatant	123

12.6. Stability

Storage Stability

Aliquots of a serum sample (spiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the IL-22 level determined after 24 h. There was significant loss of IL-22 immunoreactivity during storage at above conditions.

Freeze-thaw Stability

Aliquots of serum samples (spiked) were stored at -20°C and thawed up to 3 times, and IL-22 levels determined. There was no significant loss of IL-22 by freezing and thawing.

12.7. Normal Serum Values

Panels of 40 sera and plasma (EDTA, citrate, heparin) samples from randomly selected apparently healthy donors (males and females) were tested for human IL-22.

For detected human IL-22 levels see Table below

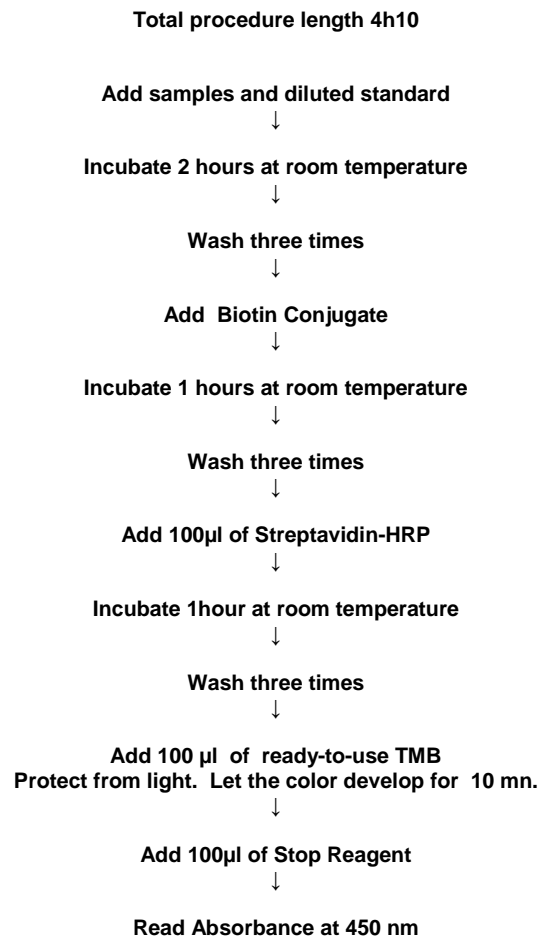
Sample Matrix	Number of Samples Evaluated	Range (pg/ml)	Mean of Detectable (pg/ml)
Serum	40	nd *- 45	3.4
Plasma (EDTA)	40	nd *- 147	21.0
Plasma (Citrate)	40	nd *- 115	12.0
Plasma (Heparin)	40	nd *- 90	9.0

* n.d. = non-detectable, samples measured below the lowest standard point are considered to be non-detectable.

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14. Assay Summary



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