Human IL-22 ELISA Kit

Instructions for use

Catalogue numbers: 1x96 tests: 650.140.096

For research use only

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Table of Contents

Human IL-22 ELISA KIT

1. Intended use

The Diaclone Human IL-22 ELISA kit is a solid phase sandwich ELISA for the *in-vitro* qualitative and quantitative determination of IL-22 in supernatants, buffered solutions or serum and plasma samples. This assay will recognise both natural and recombinant human IL-22.

This kit has been configured for research use only. Not suitable for use in therapeutic procedures.

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 coating antibody is adsorbed onto microwells. IL-22 present in the sample or standard binds to antibodies adsorbed to the microwells. Following incubation unbound biological components are removed during a wash step. A Biotin-conjugated anti-IL-22 antibody is added and binds to IL-22 captured by the first antibody.
Following incubation unbound biotin-conjugated anti-IL-22 is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-IL-22 antibody. Following incubation unbound Streptavidin-HRP is removed during a wash step and substrate solution reactive with HRP is added to the wells.
A coloured product is formed in proportion to the amount of IL-22 present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from six IL-22 standard dilutions and an IL-22 sample concentration determined.

3. Reagents provided and reconstitution

Reagents (Store@2-8ºC)	**Quantity 1x96 well kit** Cat no. 650.140.096	**Reconstitution**
Coated Plate	1	Ready to use (96 wells strip pre-coated plate)
Plastic plate covers	2	n/a
IL-22 Standard: 4 ng/ml	2	Reconstitute as directed on the vial (see Assay preparation, section 8)
Assay Buffer	1 (5ml)	20x concentrate, dilute in distilled water (see Assay preparation, section 8)
Biotin Conjugate	1 (0.14ml)	Dilute in Assay Buffer 1X (see Assay preparation, section 8)
Streptavidin-HRP	1 (0.15ml)	Dilute in Assay Buffer 1X (see Assay preparation, section 8)
Sample Diluent	1 (12ml)	Ready to use
Wash Buffer	1 (50ml)	20x concentrate, dilute in distilled water (see Assay preparation, section 8)
Substrate Solution	1 (15ml)	Ready to use
Stop Solution	1 (15ml)	Ready to use

4. Materials required but not provided

- Microtiter plate reader fitted with appropriate filters (450 nm required with optional 620 nm reference filter)
- Microtiter plate 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
- Miscellaneous laboratory plastic and/or glass, if possible sterile

5. Storage Instructions

Store kit reagents between 2 and 8°C. Immediately after use remaining reagents should be returned to cold storage (2-8°C). Expiry of the kit and reagents is stated on box front labels. The 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, the reagent is not contaminated by the first handling.

Wash Buffer 1X: Once prepared, store at 2-8°C for up to 30 days.

Assay Buffer 1X: Once prepared, store at 2-8°C for up to 30 days.

Reconstituted Standard: Once prepared use immediately and do not store.

Diluted Biotin Conjugate: Once prepared use immediately and do not store.

Diluted Streptavidin-HRP: Once prepared use immediately and do not store.

6. Specimen collection, processing & storage

Cell culture supernatants, human serum, plasma or other biological samples 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.

Cell culture supernatants: Remove particulates and aggregates by spinning at approximately 1000 x g for 10 min.

Serum: Use pyrogen/endotoxin free collecting tubes. Serum should be removed rapidly and carefully from the red cells after clotting. Following clotting, centrifuge at approximately 1000 x g for 10 min and remove serum.

Plasma: EDTA, citrate and heparin plasma can be assayed. Spin samples at 1000 x g for 30 min to remove particulates. Harvest plasma.

Storage: If not analysed shortly after collection, samples should be aliquoted (250-500µl) to avoid repeated freeze-thaw cycles and stored frozen at –70°C. Avoid multiple freeze-thaw cycles of frozen specimens.

Recommendation: Do not thaw by heating at 37°C or 56°C. Thaw at room temperature and make sure that sample is completely thawed and homogeneous before use. When possible avoid use of badly haemolysed or lipemic sera. If large amounts of particles are present these should be removed prior to use by centrifugation or filtration.

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 Stop and Substrate solutions. 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 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 Stop and Substrate solutions, 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 Substrate solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the Substrate solution with metal to prevent colour development. Warning Substrate 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 Substrate Solution has been contaminated and must be discarded. Read absorbances 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 Substrate Solution within 15 min of the washing of the microtiter 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 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	zero	zero										
H												

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

8.2. Preparation of Wash Buffer

If crystals have formed in the concentrate Wash Buffer, warm it gently until complete dissolution.

Dilute the (20X) concentrate Wash Buffer 20 fold with distilled water to give a 1X working solution.
Pour entire contents (50 ml) of the concentrate Wash Buffer 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˚-25˚C.

8.3. Preparation of Assay Buffer 1X

Dilute the (20X) concentrate Assay Buffer 20 fold with distilled water to give a 1X working solution.
Add contents of concentrate Assay Buffer (5 ml) to 95ml glass-distilled or deionized water.
Mix gently to avoid foaming and store at 2˚-25˚C.

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 4000 pg/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 62.5 pg/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 F1 and F2.
- 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 F1 and F2 providing a serial diluted standard curve ranging from 2000pg/ml to 62.5pg/ml.
- Discard 100µl from the final wells of the standard curve (F1 and F2).

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

8.5. Preparation of Biotin-Conjugate

It is recommended this reagent is prepared immediately before use. Make a 1:100 dilution of the concentrated Biotin-Conjugate with Assay Buffer 1X. Please see example volumes below:

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 1X. Please see example volumes below:

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

9. Method

We strongly recommend that every vial is mixed thoroughly without foaming prior to use.

Prepare all reagents as shown in section 8.
Note: final preparation of Biotin-Conjugate (section 8.5) and Streptavidin-HRP (section 8.6) should occur immediately before use.

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 Wash Buffer** 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 8.4 above and add in duplicate to appropriate wells
3.	Addition	Add 100µl of **Sample Diluent** in duplicate to the blank wells
4.	Addition	Add 50µl of **Sample Diluent** to sample wells
5.	Addition	Add 50µl of each **Sample** in duplicate to appropriate number of wells
6.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **2 hours** on a microplate shaker.
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 Wash Buffer** into each well c) Aspirate the contents of each well d) Repeat step b and c another two times
8.	Addition	Add 100µl of diluted **Biotin-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 hour** on a microplate shaker.
10.	Wash	Repeat wash step 7.
11.	Addition	Add 100µl of diluted **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 microplate shaker.
13.	Wash	Repeat wash step 7.
14.	Addition	Add 100µl of ready-to-use **Substrate Solution** into all wells
15.	Incubation	Incubate in the dark for **10 minutes*** at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
16.	Addition	Add 100µl of **Stop Solution** into all wells
Read the absorbance value of each well (immediately after step 16.) on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm as the reference wave length (610 nm to 650 nm is acceptable).		

**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

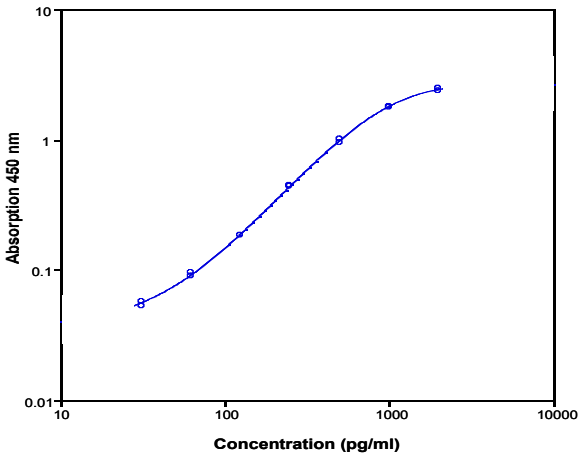
Calculate the average absorbance values for each set of duplicate standards and samples. Ideally duplicates should be within 20% of the mean.

Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding 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 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
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 human serum or plasmas 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 Assay Buffer 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 Wash Buffer, fill with Wash 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.

13. Bibliography

1- Bergman M and al, J Immunol May 1, 1996, 156 (9) 3118-3132
2- Brugnoni D and al, Haematologica Vol. 80 No. 5 (1995): September, 1995
3- Cosman D, Stem Cells. 1994 Sep;12(5):440-55
4- Durie F and al, Science. 1993 Sep3; 261(5126): 1328-30
5- Holder-MJ and al, Eur-J-Immunol. 1993 Sep; 23(9): 2368-71
6- Lane-P and al, J-Exp-Med. 1993 Apr 1; 177(4): 1209-13
7- Mazzei-GJ and al, J-Biol-Chem. 1995 Mar 31; 270(13): 7025-8
8- Rathmell J and al, Cell, Vol. 87, 319-329, October 18, 1996
9- Rothstein-T and al, Nature. 1995 Mar 9; 374(6518): 163-5
10- Tsubata-T and al, Philos-Trans-R-Soc-Lond-B-Biol-Sci. 1994 Aug 30; 345(1313): 297-301

14. Assay Summary

Total procedure length : 4h10mn

Add Samples and Standards

↓

Incubate 2 hours at room temperature

↓

Wash three times

↓

Add 100µl of diluted Biotin-Conjugate

↓

Incubate 1 hour at room temperature

↓

Wash three times

↓

Add 100µl of diluted Streptavidin-HRP

↓

Incubate 30 min at room temperature

↓

Wash three times

↓

Add 100µl of Substrate Solution
Protect from light. Let the color develop for 10 min.

↓

Add 100µl of Stop Solution

↓

Read Absorbance at 450 nm

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