Murine IL-22 ELISA Kit

Instructions for use

Catalogue numbers: 1x96 tests: 660.110.096

For research use only

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1. Intended use

The mouse IL-22 ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of mouse IL-22. **The mouse IL-22 ELISA is for research use only. Not for diagnostic or 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.
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2.2. Principle of the method

An anti-mouse IL-22 coating antibody is adsorbed onto microwells.

Mouse IL-22 present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-mouse IL-22 antibody is added and binds to mouse IL-22 captured by the first antibody.
Following incubation unbound biotin-conjugated anti-mouse IL-22 antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-mouse 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 mouse 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 7 mouse IL-22 standard dilutions and mouse IL-22 sample concentration determined.

3. Reagents provided and reconstitution

REAGENTS (store at 2-8°C)	Quantity 1x96 well-kit 660.110.096	RECONSTITUTION
96-wells precoated microtiter plate	1	Ready-to-use
Plate covers	2	
Biotin-Conjugate anti-mouse IL-22 antibody	1 vial	Dilute 100 times in Assay Buffer (70µl)
Streptavidin-HRP	1 vial	Dilute 200 times in Assay Buffer (150µl)
mIL-22 Standard: 500 pg/ml	2 vials	Reconstitute as indicated on the vial
Sample Diluent	1 vial	(12 ml) Ready-to-use
Calibrator Diluent	1 vial	(5 ml) Ready-to-use
Assay Buffer Concentrate	1 vial	(5 ml) 20X concentrate. Dilute in distilled water
Wash Buffer Concentrate	1 vial	(50 ml) 20X concentrate. Dilute in distilled water
Substrate Solution	1 vial	(15 ml) Ready-to-use
Stop Solution (1M Phosphoric acid)	1 vial	(15 ml) Ready-to-use

4. Materials required but not provided

- Microtitre plate reader fitted with appropriate filters (450nm required with optional 620nm 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
- 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° to 8°C). 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 supernatant, serum and plasma (citrate) were tested with this assay. Other body fluids might be suitable for use in the assay. Remove serum from the clot as soon as possible after clotting.

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 aliquoted and must be stored frozen at -20°C to avoid loss of bioactive mouse IL-22. If samples are to be run within 24 hours, they may be stored at 2° to 8°C.
Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample 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 H_2SO_4 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 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 H_2SO_4 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 microtiter plate.

8. Assay Preparation

Bring all reagents to room temperature before use
If crystals have formed in the **Buffer Concentrates**, warm them gently until they have completely dissolved.

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	500	500										
B	250	250										
C	125	125										
D	62.5	62.5										
E	31.2	31.2										
F	15.6	15.6										
G	Zero	Zero										
H												

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

8.2. Preparation of Wash Buffer

Pour entire contents (50 ml) of the **Wash Buffer Concentrate** (20x) into a clean 1000 ml graduated cylinder. Bring to final volume of 1000 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 Wash Buffer (1x) is stable for 30 days.
Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

8.3. Preparation of Assay Buffer

Pour the entire contents (5 ml) of the **Assay Buffer Concentrate** (20x) into a clean 100 ml graduated cylinder. Bring to final volume of 100 ml with distilled water. Mix gently to avoid foaming.
Store at 2° to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.
Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

8.4. Preparation of Standard

Reconstitute **mouse IL-22 standard** by addition of Calibrator Diluent.

Reconstitution volume is stated on the label of the standard vial. Allow the reconstituted standard to sit for 10-30 minutes. Swirl or mix gently to insure complete and homogeneous solubilisation (concentration of reconstituted standard = 500 pg/ml).

Label 6 tubes, one for each standard point. S2, S3, S4, S5, S6, S7.
The reconstituted standard serves as S1.
Then prepare 1:2 serial dilutions for the standard curve as follows:
Pipette 150 µl of Calibrator Diluent into each tube S2-S7.
Pipette 150 µl of reconstituted standard (concentration = 500 pg/ml) into the tube, labelled S2, and mix (concentration of standard 2 = 250 pg/ml).
Pipette 150 µl of this dilution into the second tube, labelled S3, and mix thoroughly before the next transfer.
Repeat serial dilutions 4 more times thus creating the points of the standard curve ranging from 500 to 7.8pg/ml.
Calibrator Diluent serves as blank.

8.5. Preparation of Biotin-Conjugate

Please note that the Biotin-Conjugate should be used within 30 minutes after dilution.

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

Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (1x) (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

8.6. Preparation of Streptavidin-HRP

Please note that the Streptavidin-HRP should be used within 30 minutes after dilution.

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

Number of Strips	Streptavidin-HRP (ml)	Assay Buffer (1x) (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	a) Dispense 0.4 ml of **1x washing solution** into each well b) Aspirate the contents of each well c) Repeat steps a and b Do not allow wells to dry before use
2.	Addition	Add 50µl of **Sample diluent** to all wells
3.	Preparation	**Prepare Standard curve** as shown in section 8.4
4.	Addition	Add 50µl of each **Standard, Sample and Blank (Calibrator Diluent)** in duplicate to appropriate number of wells
5.	Addition	Add 50µl of diluted **biotin-conjugate** to all wells
6.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **2 hour(s)** if available on a microplate shaker set at 400 rpm
7.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well b) Dispense 0.4 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 100µl of **Streptavidin-HRP** solution into all wells
9.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **1 hour** if available on a microplate shaker set at 400 rpm
10.	Wash	Repeat wash step 7.
11.	Addition	Add 100µl of ready-to-use **TMB Substrate Solution** into all wells
12.	Incubation	Incubate in the dark for **30 minutes*** at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
13.	Addition	Add 100µl of **Stop Solution** into all wells
Read the absorbance value of each well (immediately after step 13.) 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.

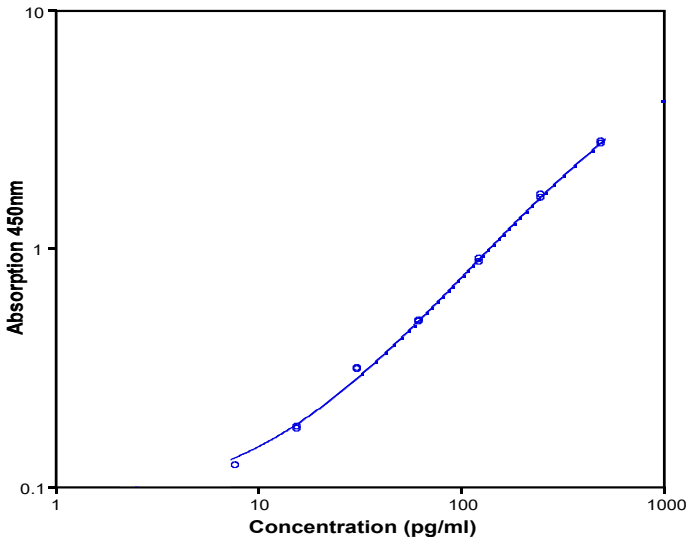
Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding mIL-22 standard concentration on the horizontal axis.

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

Example mIL-22 Standard Curve:

Standard	mIL-22 Conc	OD (450nm) mean	CV (%)
1	500.0	2.305	0.2
2	250.0	1.436	2.7
3	125.0	0.744	0.8
4	62.5	0.442	3.9
5	31.3	0.254	1.3
6	15.6	0.162	1.4
Blank	0.0	0.046	2.0

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.



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 mouse IL-22 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 5.0 pg/ml (mean of 6 independent assays).

12.2. Specificity

The assay detects both natural and recombinant mouse IL-22.
The cross reactivity and interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations in a mouse IL-22 positive sample.
There was no cross reactivity or interference detected.

12.3. Precision

Intra Assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum and plasma samples containing different concentrations of mouse IL-22. 2 standard curves were run on each plate. **The calculated overall intra-assay coefficient of variation was 3.6%.**

Inter-assay
Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum and plasma samples containing different concentrations of mouse IL-22. 2 standard curves were run on each plate. **The calculated overall inter-assay coefficient of variation was 5.8%.**

12.4. Dilution Parallelism

Serum, plasma and cell culture supernatant samples with different levels of mouse IL-12 were analyzed at serial 2 fold dilutions (1:4 to 1:16) with 4 replicates each. Recoveries ranged from 91 to 129% with an overall **mean recovery of 104%.**

12.5. Spike recovery

The spike recovery was evaluated by spiking 3 levels of mouse IL-22 into serum, plasma (citrate) and cell culture supernatant. Recoveries were determined with 2 replicates each. The amount of endogenous mouse IL-22 in unspiked samples was substracted from the spike values. Recoveries ranged from 73 to 98% with an overall **mean recovery of 88%.**

12.6. Stability

Freeze-Thaw Stability

Aliquots of serum and plasma samples (spiked or unspiked) were stored at -20°C and thawed 3 times, and the mouse IL-22 levels determined. There was no significant loss of mouse IL-22 immunoreactivity detected by freezing and thawing.

Storage Stability

Aliquots of serum and plasma samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the mouse IL-22 level determined after 24 h. There was no significant loss of mouse IL-22 immunoreactivity detected at -20°C and 2-8°C.
A significant loss of mouse IL-22 immunoreactivity was detected during storage at room temperature (RT) and at 37°C after 24 hours.

12.7. Expected Values

A panel of samples from randomly selected healthy donors (males and females) was tested for mouse IL-22. See results below:

Sample Matrix	Number of samples evaluated	Range (pg/ml)	Mean (pg/ml)	Standard deviation (pg/ml)
Serum	8	13.0 – 267.0	121.9	84.0
Plasma (citrate)	4	73.5 – 191.8	123.2	56.7

13. Assay Summary

Total procedure length : 3h30mn

Wash two times

↓

Add sample, diluted standard and Biotin-Conjugate

↓

Incubate 2 hours at room temperature

↓

Wash three times

↓

Add 100µl of Streptavidin-HRP

↓

Incubate 1hour at room temperature

↓

Wash three times

↓

Add 100 µl of ready-to-use TMB
Protect from light. Let the color develop for 30 mn.

↓

Add 100 µl Stop Solution

↓

Read Absorbance at 450 nm

Supplier:

Diaclone SAS
6 Rue Dr Jean-François-Xavier Girod
25000 Besançon
France
Tel +33 (0)3 81 41 38 38
Email: techsupport@medixbiochemica.com