

Human IL-22 ELISA Kit

Catalog No.: CDK065A, B **Lot No.:** TBD **Size:** 1 or 2 Plates (1 or 2 x 96 tests) **Expiration date:** TBD

Specificity:	Recognizes both native and recombinant human IL-22
Sensitivity:	5.0 pg / mL
Range:	62.5 pg / mL – 2000 pg / mL
Sample Type:	Buffered solutions, cell culture supernatant, serum and plasma samples.

1. PRINCIPLE OF THE METHOD

A capture antibody highly specific for IL-22 has been coated to the wells of the microtiter strip plate during manufacture. Binding of IL-22 in samples and known standards to the capture antibodies and subsequent binding of the biotinylated anti-IL-22 secondary antibody to the analyte is completed during the same incubation period. Any excess unbound analyte and secondary antibody is then removed. The HRP conjugate solution is then added to every well including the zero wells. Following incubation, excess conjugate is removed by careful washing. A chromogen substrate is added to the wells resulting in the progressive development of a blue colored complex with the conjugate. The color development is then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced colored complex is directly proportional to the concentration of IL-22 present in the samples and standards. The absorbance of the color complex is then measured and the generated OD values for each standard are plotted against expected concentration forming a standard curve. This standard curve can then be used to accurately determine the concentration of IL-22 in any sample tested.

2. REAGENTS PROVIDED AND RECONSTITUTION

REAGENTS	Quantity (1 x 96 wells)	Quantity (2 x 96 wells)	RECONSTITUTION
CDK065-P: 96-well Coated plate	1	2	Ready-to-use strips (pre-coated).
CDK065-Z: Plastic plate covers	2	4	n/a
CDK065-A: IL-22 Standard: 4 ng/mL	2 vials	4 vials	Resuspend as directed on the vial.
CDK065-B: Assay Buffer	1 (5 mL)	2 (5 mL)	20x Concentrate, dilute in distilled water. (see Assay preparation)
CDK065-C: Biotinylated anti-IL-22	1 (0.14mL)	2 (0.14mL)	Dilute in 1X Assay Buffer (see Assay preparation)
CDK065-D: Streptavidin-HRP	1 (0.15 ml)	2 (0.15 ml)	Dilute in 1X Assay Buffer (see Assay preparation)
CDK065-E: Sample Diluent	1 (12 mL)	2 (12 mL)	Ready-to-use.
CDK065-F: Wash Buffer	1 (50 mL)	2 (50 mL)	20x concentrate dilute in distilled water. (see Assay preparation)
CDK065-G: TMB Substrate	1 (15 mL)	1 (15 mL)	Ready-to-use.
CDK065-H: H ₂ SO ₄ : Stop Reagent	1 (15 mL)	2 (15 mL)	Ready-to-use.



Human IL-22 ELISA Kit

3. STORAGE INSTRUCTIONS

Store kit reagents between 2-8 °C. Immediately after use remaining reagents should be returned to cold storage (2-8 °C). Expiration of the kit and reagents is stated on box front labels. The expiration of the kit components can only be guaranteed if the components are stored properly, and if the reagent is not contaminated by handling.

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

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

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.

4. MATERIALS REQUIRED BUT NOT PROVIDED

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

5. SPECIMEN COLLECTION, PROCESSING AND STORAGE

Cell culture supernatants, human serum, plasma or other biological samples are suitable for use in the assay.

Serum: Remove serum from the clot or red cells as soon as possible after clotting and separation, respectively.

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

Plasma: EDTA, citrate and heparin plasma may be assayed following centrifugation at approximately 1000 x g for 30 minutes to remove particulates. Remove clear plasma.

If not analyzed shortly after collection, samples should be aliquoted (250-500 µL) to avoid repeated freeze-thaw cycles and stored frozen at -80 °C. Avoid multiple freeze-thaw cycles of frozen specimens. Do not thaw by heating at 37 °C or 56 °C. Thaw at room temperature and make sure that the sample is completely thawed and homogeneous before use. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particles are present these should be removed prior to use by centrifugation or filtration.



Human IL-22 ELISA Kit

6. 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" 2009.
- The human serum included in this kit have been tested and found non-reactive for HBsAg, anti-HIV1 & 2, and anti-HCV. Nevertheless, no known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.
- 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 vial or bottle 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.
- 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 color development. **Warning TMB is toxic avoid direct contact with hands. Dispose of properly.**
- If a dark blue color 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.
- Maintain a consistent order of addition of reagents to wells to ensure equal incubation times for all wells.
- Dispense the TMB solution within 15 minutes of the washing of the microtiter plate.

7. ASSAY PREPARATION

Bring all reagents to room temperature before use.

7.1 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 aluminum 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.*



Human IL-22 ELISA Kit

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

7.2 Preparation of Wash Buffer 1X

Dilute the **(20x) Wash Buffer concentrate** 20-fold with distilled water to give a 1X working solution. Pour entire contents (50 mL) of the Washing 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.

Store Wash Buffer 1X at 2°-8 °C for up to 1 week.

7.3 Preparation of Assay Buffer 1X

Add the contents of the **20X concentrate Assay Buffer** (5 ml) to 95 mL of distilled water before use. Mix gently.

Assay Buffer 1X can be stored at 2-8 °C for up to 1 week.

7.4 Preparation of Standard

Resuspend each Standard vial, immediately prior to use with **the volume of distilled water indicated on the vial**. 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 of 2000 to 62.5 pg/mL. A fresh standard curve should be produced for each new assay.

- Immediately after reconstitution add 100 µL of the reconstituted standard to wells A1 and A2, which provides the highest concentration standard at 2000 pg/mL.
- Add 100 µL of **Sample Diluent** to the remaining standard wells B1 and B2 to F1 and F2.
- Transfer 100 µL 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 100 µL from wells B1 and B2 through to wells F1 and F2 providing a serial diluted standard curve ranging from 2000 pg/ml to 62.5 pg/mL.
- Discard 100 µL from the final wells of the standard curve (F1 and F2).

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



Human IL-22 ELISA Kit

7.5 Preparation of Samples

Before testing, serum or plasmas samples should be diluted at least 1:2 in Sample Diluent.

7.6 Preparation of Biotinylated anti-IL-22

It is recommended this reagent is prepared immediately before use. Make a 1:100 dilution of the concentrated Biotin anti-IL-22 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

7.7 Preparation of Streptavidin-HRP

It is recommended to centrifuge vial for a few seconds in a micro centrifuge to collect all the volume at the bottom.

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

8. 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 instructed in section 7.

Note: final preparation of Biotinylated anti-IL-22 (section 7.6) and Streptavidin-HRP (section 7.7) should occur immediately before use.

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



Human IL-22 ELISA Kit

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
<p>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).</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 color development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range.



Human IL-22 ELISA Kit

9. DATA ANALYSIS

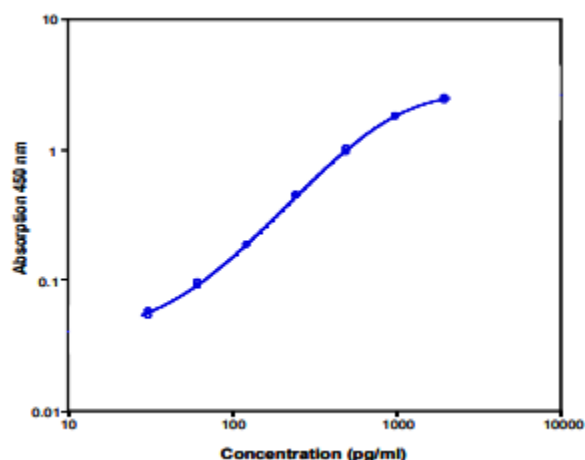
Calculate the average absorbance values for each set of duplicate standards and samples. Ideally duplicates should be within 20% of the mean.

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 Concentration (pg/ml)	OD (450 nm) 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 is an example only and should not be used to determine results. Every laboratory must produce a standard curve for each set of microwell strips assayed.

10. 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 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.



Human IL-22 ELISA Kit

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.**

11. PERFORMANCE CHARACTERISTICS

11.1 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).

11.2 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.

11.3 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%.

11.4 Dilution Parallelism

Serum, plasma and cell culture supernatant samples with different levels of human IL-22 were analyzed 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



Human IL-22 ELISA Kit

11.5 Spike Recovery

The spike recovery was evaluated by spiking three concentrations of recombinant mIL-22 in mouse serum and culture media in two experiments. Recoveries ranged from 68% to 103% with an overall **mean recovery of 85% when spiked in serum**. Recoveries ranged from 114% to 126% with an overall **mean recovery of 121% when spiked in culture media**.

11.6 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.

11.7 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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