

TiterZyme[®] EIA

human IL-4

Enzyme Immunometric Assay Kit

Catalog No. 900-145

96 Well Kit

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Description

Assay Designs' human IL-4 TiterZyme® Enzyme Immunometric Assay (EIA) kit is a complete kit for the quantitative determination of human IL-4 in biological fluids. Please read the complete kit insert before performing this assay. The kit uses an antibody to human IL-4 immobilized on a microtiter plate to bind the human IL-4 in the standards or sample. A recombinant human IL-4 Standard is provided in the kit. A biotinylated antibody to human IL-4 is incubated with the standards and samples in the plate. This antibody binds to the human IL-4 captured on the plate. After a short incubation the excess standards, samples and antibody are washed out and streptavidin conjugated to Horseradish peroxidase is added, which binds to the biotinylated human IL-4 antibody. Excess conjugate is washed out and substrate is added. After a short incubation, the enzyme reaction is stopped and the color generated is read at 450 nm. The measured optical density is directly proportional to the concentration of human IL-4 in either standards or samples. For further explanation of the principles and practices of immunoassays please see the excellent books by Chard¹ or Tijssen².

Introduction

Human Interleukin-4 (h IL-4) is an anti-inflammatory glycoprotein of 129 amino acids with a molecular mass of 15-19 kDa.^{3,4} It has also been referred to as B cell growth factor (BCGF)-1, B cell stimulating factor (BSF), mast cell growth factor, and T cell growth factor (TCGF)-II.^{5,6} IL-4 is a co-stimulator of B-cell proliferation, as well as a growth factor for T cells.⁹ IL-4 regulates cytokine production through T, B, and NK cells, as well as monocytes, macrophages, fibroblast, and endothelial cells.⁵ Its inhibitory effects on cytokine production make it a potential candidate for use in chronic inflammatory disease.³ There is also a prospect that IL-4 may play a role in antitumor activity.^{3,7,8} There is a 50% amino acid level homology shared by human and mouse IL-4.

Precautions

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1. Stop Solution is a 0.18 M sulfuric acid solution. This solution is caustic; care should be taken in use.
2. The activity of the Horseradish peroxidase conjugate is affected by nucleophiles such as azide, cyanide and hydroxylamine.
3. We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results.
4. The human IL-4 Standards provided, Catalog No.80-1389, should be handled with care because of the known and unknown effects of IL-4.

Materials Supplied

1. **human IL-4 Microtiter Plate, One Plate of 96 Wells, Catalog No. 80-1385**
A plate using break-apart strips coated with antibody specific to human IL-4.
2. **human IL-4 Antibody, 8 mL, Catalog No. 80-1388**
A solution of biotinylated monoclonal antibody to human IL-4.
3. **human IL-4 Standard Diluent, 14 mL, Catalog No. 80-1386**
4. **human IL-4 Streptavidin-HRP Concentrate, 75 μ L, Catalog No. 80-1387**
A concentrated solution of streptavidin conjugated to Horseradish peroxidase.
5. **human IL-4 Streptavidin-HRP Dilution Buffer, 13 mL, Catalog No. 80-1391**
6. **Wash Buffer Concentrate, 50 mL, Catalog No. 80-1253**
Tris buffered saline containing detergents.
7. **human IL-4 Standard, 2 vials, Catalog No. 80-1389**
Two vials of lyophilized recombinant human IL-4.
8. **human IL-4 TMB Substrate, 12 mL, Catalog No. 80-1390**
A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide. Ready to use.
Protect from prolonged exposure to light.
9. **human IL-4 Stop Solution, 13 mL, Catalog No. 80-1392**
A 0.18 M solution of sulfuric acid in water. Keep tightly capped. Caution: **Caustic.**
10. **human IL-4 Assay Layout Sheet, 1 each, Catalog No. 30-0223**
11. **Plate Sealer, 2 each, Catalog No. 30-0012**

Storage

All components of this kit are stable at 4 °C until the kit's expiration date.

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Precision pipets for volumes between 50 μ L and 1,000 μ L.
3. Disposable polypropylene or polyethylene test tubes for dilution of samples and standards.
4. Repeater pipets for dispensing 50 μ L and 100 μ L.
5. Disposable beakers for diluting buffer concentrates.
6. Graduated cylinders.
7. Microcentrifuge to prepare Streptavidin-HRP Solution.
8. Adsorbent paper for blotting.
9. Microplate reader capable of reading at 450 nm, preferably with correction between 570 nm and 590 nm.
10. Graph paper for plotting the standard curve.

Sample Handling

Assay Designs' TiterZyme[®] EIA is compatible with human IL-4 samples in a wide range of matrices. Samples diluted sufficiently into the proper diluent can be read directly from a standard curve.

Culture fluids, serum, plasma (EDTA, heparin, and sodium citrate), and urine are suitable for use in the assay. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples in the majority of Tissue Culture Media can also be read in the assay, provided the standards have been diluted into the Tissue Culture Media instead of Standard Diluent. There will be a small change in binding associated with running the standards and samples in media. Users should only use standard curves generated in media, or buffer to calculate concentrations of human IL-4 in the appropriate matrix.

If samples are to be run within 24 hours, they may be stored at 4 °C. Otherwise, samples must be stored frozen at -70 °C to avoid loss of bioactive human IL-4. Excessive freeze/thaw cycles should be avoided. Prior to assay, frozen sera should be brought to room temperature slowly and gently mixed by hand. Do not thaw samples in a 37 °C incubator. Do not vortex or sharply agitate samples.

Procedural Notes

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards must be prepared in polypropylene or polyethylene tubes. Do not use polystyrene, polycarbonate or glass tubes.
4. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. This kit uses plates with removable strips. Unused wells must be kept desiccated at 4 °C in the sealed bag provided. The strips should be used in the frame provided.
8. **Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.**
9. **It is important that the matrix for the standards and samples be as similar as possible. Human IL-4 samples diluted with Standard Diluent should be run with a standard curve diluted in the same buffer. Serum, plasma and urine samples should be evaluated against a standard curve run in Standard Diluent while tissue culture samples should be read against a standard curve diluted in the same complete but non-conditioned media. See Reagent Preparation, step #2.**

Reagent Preparation

1. Wash Buffer

Prepare the Wash Buffer by diluting 50 mL of the supplied concentrate with 1,450 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. human IL-4 Standards

Reconstitute standard with deionized water. Reconstitution volume is stated on the standard vial label. Let it sit at room temperature for 5 minutes. Mix gently. This solution contains 1,000 pg/mL human IL-4. When testing serum, plasma or urine samples, use the Standard Diluent provided to prepare standard curve serial dilutions. When using cell culture supernatants, use tissue culture media to prepare the standard curve serial dilutions.

Label five 12x75 mm test tubes #1 through #5. Pipet 240 µL of Standard Diluent or tissue culture media into tubes #1 through #5. Add 160 µL of the 1,000 pg/mL Standard to tube #1. Vortex thoroughly. Add 160 µL of tube #1 to tube #2 and vortex thoroughly. Add 160 µL of tube #2 to tube #3 and vortex thoroughly. Continue this for #4 and #5.

The concentration of human IL-4 in tubes #1 through #5 will be 400, 160, 64, 25.6, and 10.24 pg/mL, respectively. See human IL-4 Assay Layout Sheet for dilution details.

Diluted standards should be used within 60 minutes of preparation. Do not store reconstituted standards.

3. Streptavidin-HRP Solution

Prepare Streptavidin-HRP solution **immediately before use**. Do not store prepared Streptavidin-HRP solution. Use a plastic tube to prepare Streptavidin-HRP solution. Briefly centrifuge the Streptavidin-HRP Concentrate to force entire vial contents to the bottom. For each strip used, mix 2.5 μL of Streptavidin-HRP Concentrate with 1 mL of Streptavidin-HRP Dilution Buffer.

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards, controls and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4 °C.
2. Pipet 50 μL of the Antibody into each well, except the Blank.
3. Pipet 50 μL of Standard Diluent or Tissue Culture Media into the S0 (0 pg/mL standard) wells.
4. Pipet 50 μL of Standards #1 through #5 into the appropriate wells.
5. Pipet 50 μL of the Samples into the appropriate wells.
6. Tap the plate gently to mix the contents.
7. Seal the plate and incubate at room temperature for 2 hours.
8. Empty the contents of the wells and wash by adding 400 μL of wash solution to every well. Repeat the wash 2 more times for a total of **3 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
9. Pipet 100 μL of prepared Streptavidin-HRP Solution into each well, except the Blank.
10. Seal the plate and incubate at room temperature for 30 minutes.
11. Empty the contents of the wells and wash by adding 400 μL of wash solution to every well. Repeat the wash 2 more times for a total of **3 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
12. Pipet 100 μL of Substrate Solution into each well.
13. Incubate for 1 hour at room temperature in the dark.
14. Pipet 100 μL Stop Solution to each well. This stops the reaction and the plate should be read immediately.
15. Blank the plate reader against the Blank wells, read the optical density at 450 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all the readings.

Calculation of Results

Several options are available for the calculation of the concentration of human IL-4 in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of human IL-4 can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD for each standard and sample.

$$\text{Average Net OD} = \text{Average OD} - \text{Average Blank OD}$$

2. Using linear graph paper, plot the Average Net OD for each standard versus human IL-4 concentration in each standard. Approximate a straight line through the points. The concentration of human IL-4 in the unknowns can be determined by interpolation.

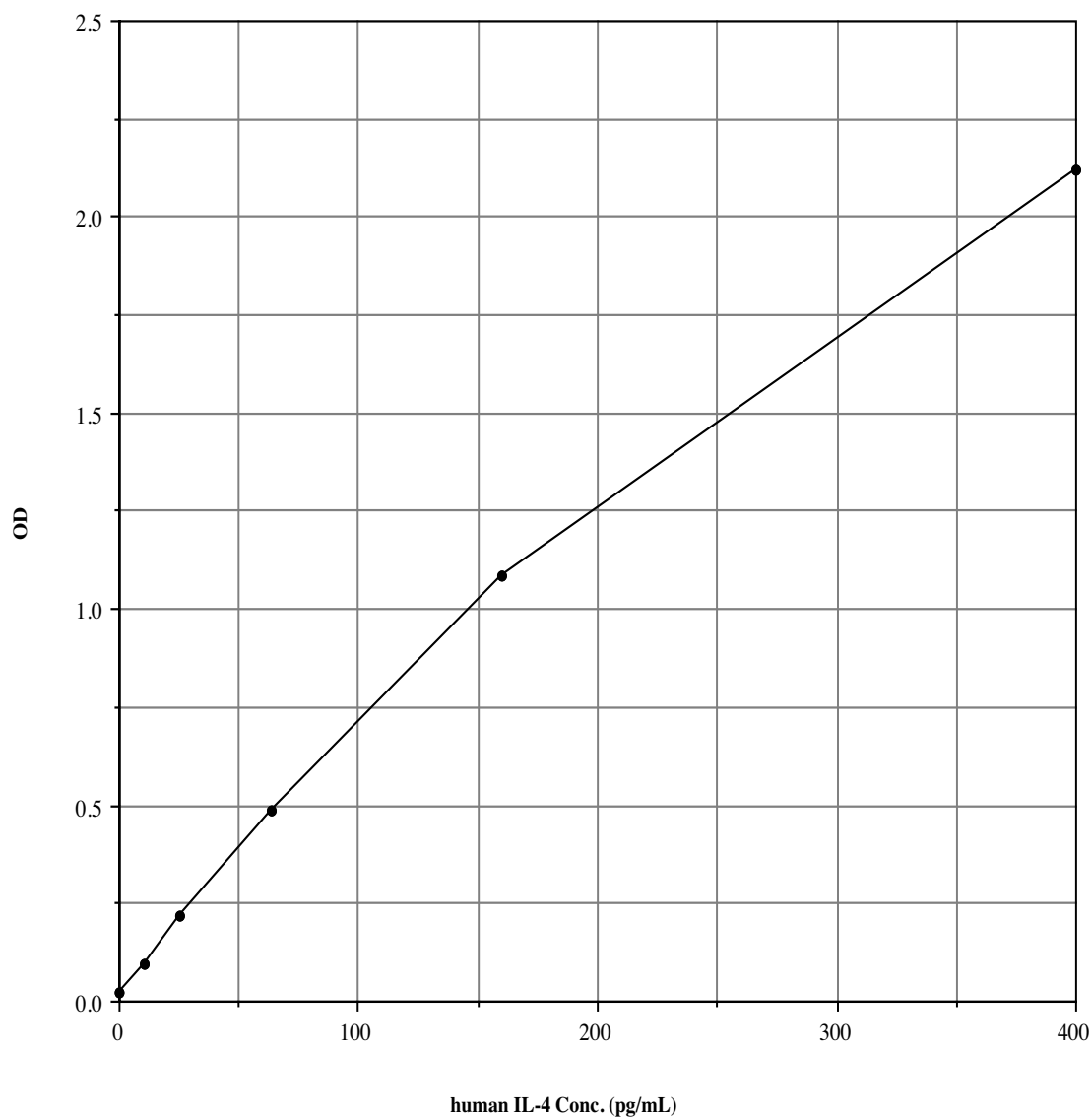
Typical Results

The results shown below are for illustration only and **should not** be used to calculate results from another assay.

<u>Sample</u>	<u>Average OD</u>	<u>Net OD</u>	<u>h IL-4 (pg/mL)</u>
Blank	(0.048)		
S0	0.072	0.024	0
S1	2.170	2.122	400
S2	1.132	1.084	160
S3	0.537	0.489	64
S4	0.264	0.216	25.6
S5	0.145	0.097	10.24
Unknown 1	0.450	0.402	51.5
Unknown 2	0.155	0.107	11.3

Typical Standard Curve

Typical standard curves are shown below. These curves **must not** be used to calculate human IL-4 concentrations; each user must run a standard curve for each assay.



Calibration:

The standards in this assay have been re-calibrated to the NIBSC reference standard lot 88/656. One (1) pg of Assay Designs' standard = 6.4 NIBSC, pg = 0.064 NIBSC units.

Performance Characteristics

Sensitivity: < 2 pg/mL

The sensitivity or Lower Limit of Detection (LLD) is determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 standard deviations read in dose from the standard curve is the LLD. This value is the smallest dose that is not zero with 95% confidence.

Linearity

Linearity of dilution was determined by serially diluting six different positive samples. The dilutions were evaluated in the assay and the “found” doses are plotted against the “expected” doses.

The line obtained had a slope of 0.985 with a correlation coefficient of 0.998.

Precision

Intra-Assay CV: < 10%

Inter-Assay CV: < 10%

Cross Reactivities

The TiterZyme® human IL-4 EIA Kit is specific for native and recombinant human IL-4. It is unaffected by the presence of the following recombinant molecules: human IL-2, IL-6, IL-10, IFN- γ , GM-CSF, TNF- α , TNF- β , or mouse IL-4.

Sample Recoveries

Please refer to pages 4 and 5 for Sample Handling recommendations and Standard preparation.

Human IL-4 was not detected in eight serum samples, eight plasma samples and five urine samples collected from healthy individual donors.

<u>Spike Level</u>	<u>30 pg/mL</u>	<u>100 pg/mL</u>	<u>200 pg/mL</u>
Serum Recovery	116%	104%	114%
Plasma Recovery	107%	105%	111%
Urine Recovery	100%	103%	98%

References

1. T. Chard, in "An Intro. to Radioimmunoassay & Related Tech.", (1990), 4th Ed., Elsevier, Amsterdam.
2. P. Tijssen, in "Practice & Theory of Enz. Immunoassays", (1985), Elsevier, Amsterdam.
3. Chomarat P, Rybak ME, Banchereau J Thomson A, editors. "The Cytokine Handbook." (1998), Academic Press, pp 133-74.
4. Yokota T, et al., Proc Natl Acad Sci USA. (1986), 83,5894-5898.
5. Park LS, et al., J Exp Med. (1987), 166:476-88.
6. Boulay JL, et al., Cur Opin Immunol. (1992), 4(3):294-8.
7. Kim YS, et al., Gene Ther. (2000), 7(10):837-43.
8. Rodolfo M, et al., J Immunol. (1999), 163(4):1923-8.
9. Whicher JT, et al., Clin Chem., (1990), 36(7):1269-1281.
10. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, 1989, NCCLS, Villanova, PA, 19085.

LIMITED WARRANTY

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Material Safety Data Sheet (MSDS) available on our website or by fax.

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