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TiterZyme ®EIA

human IL-5

Enzyme Immunometric Assay Kit

Catalog No. 900-129

96 Well Kit

Table of Contents

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Description

Assay Designs' human IL-5 TiterZyme® Enzyme Immunometric Assay (EIA) kit is a complete kit for the quantitative determintation of human IL-5 in biological fluids. Please read the complete kit insert before performing this assay. The kit uses a monoclonal antibody to human IL-5 immobilized on a microtiter plate to bind the human IL-5 in the standards or sample. A recombinant human IL-5 Standard is provided in the kit. After a short incubation the excess sample or standard is washed out and a biotinylated monoclonal antibody to human IL-5 is added. This antibody binds to the human IL-5 captured on the plate. After a short incubation the excess antibody is washed out and streptavidin conjugated to Horseradish peroxidase is added, which binds to the biotinylated human IL-5 antibody. Excess conjugate is washed out and substrate is added. The substrate reacts with the conjugate bound to the IL-5 captured on the plate. 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-5 in either standards or samples. For further explanation of the principles and practices of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

Introduction

Interleukin-5 (IL-5) is produced by lymphocytes as a glycosylated, disulfide-linked homodimer[3]. IL-5 is the only eosinophil haematopoietic growth factor that cross-reacts in both human and mouse species[4]. While initially identified by a number of groups based on different biochemical aspects, IL-5 is now known to be the predominant cytokine in eosiphilia without influencing IgE response (a function of IL-4). The genes encoding IL-4 and IL-5 are tightly linked and the proteins are frequently co-expressed although they are under the control of unrelated promoters[5]. IL-5 belongs to a family of structurally related cytokines including IL-2, IL-4, macrophage colony stimulating growth factor MG-CSF and growth hormone. The major role that eosinophils occupy in the development of chronic allergy repsonse makes IL-5 a primary target for the development of next generation anti-allergy drugs[6,7], IL-5 is also a potential marker of acute graft-versus-host disease[8]. T cells, mast cells, adult T cell leukemia cell lines and Epstein-Barr virus-transformed B cells, produce IL-5. Mouse and human IL-5 share a 71% homology at the amino acid level[9].

Precautions

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1. Stop Solution 2 is a 1 normal (1N) hydrochloric 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-5 Standard provided, Catalog No. 80-1226, should be handled with care because of the known and unknown effects of IL-5.

Materials Supplied

1. **human IL-5 Microtiter Plate, One Plate of 96 Wells, Catalog No. 80-1221**
   A strip microtiter plate coated with monoclonal antibody specific to human IL-5.
2. **human IL-5 Antibody , 8.5 mL, Catalog No. 80-1222**
   A solution of biotinylated monoclonal antibody to human IL-5.
3. **Standard Diluent, 12.5 mL, Catalog No. 80-1223**
   Phosphate buffered saline containing proteins and antibiotics.
4. **human IL-5 Streptavidin-HRP Concentrate, 80 µL, Catalog No. 80-1224**
   A concentrated solution of streptavidin conjugated to Horseradish peroxidase.
5. **Streptavidin-HRP Dilution Buffer, 14.5 mL, Catalog No. 80-1225**
6. **Wash Buffer Concentrate, 30 mL, Catalog No. 80-1286**
   Tris buffered saline containing detergents.
7. **human IL-5 Standard, 2 vials, Catalog No. 80-1226**
   Two vials of lyophilized recombinant human IL-5.
8. **TMB Substrate, 11 mL, Catalog No. 80-0350**
   A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide. **Protect from prolonged exposure to light.**
9. **Stop Solution 2, 11 mL, Catalog No. 80-0377**
   A 1N solution of hydrochloric acid in water. Keep tightly capped. Caution: **Caustic.**
10. **human IL-5 Assay Layout Sheet, 1 each, Catalog No. 30-0205**
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. No difference in assay results is seen with distilled water.
2. Precision pipets for volumes between 50 µL and 1,000 µL.
3. Repeater pipet for dispensing 50 and 100 µL.
4. Disposable beakers for diluting buffer concentrates.
5. Graduated cylinders.
6. Adsorbent paper for blotting.
7. Microcentrifuge to prepare Streptavidin-HRP Solution.
8. Microplate reader capable of reading at 450 nm, preferably with correction between 570 nm and 590 nm.
9. Log-log graph paper for plotting the standard curve.

Sample Handling

Assay Designs' TiterZyme® Enzyme Immunometric Assay is compatible with human IL-5 samples in a wide range of matrixes. Samples diluted sufficiently into the proper diluent (≥ 1:5) can be read directly from a standard curve.

Culture fluids, serum, EDTA, heparin and sodium citrate plasma 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 Standard Diluent to calculate concentrations of human IL-5 in the appropriate matrix.

Samples must be stored frozen to avoid loss of bioactive human IL-5. If samples are to be run within 24 hours, they may be stored at 4 °C. Otherwise, samples must be stored frozen at or below -70 °C to avoid loss of bioactive human IL-5. Up to three freeze/thaw cycles of serum has been shown to have no effect on human IL-5 levels. Nonetheless, 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 strips must be kept desiccated at 4 °C in the sealed bag provided. The wells should be used in the frame provided.
8. **Prior to addition of antibody, conjugate and 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-5 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 Preperation, step #2.**

Reagent Preparation

1. Wash Buffer

Prepare the Wash Buffer by diluting 30 mL of the supplied concentrate with 570 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-5 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-5. 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 #3 and vortex thoroughly. Continue this for tubes #4 and #5.

The concentration of human IL-5 in tubes #1 through #5 will be 400, 160, 64, 25.6 and 10.24 pg/mL respectively. See human IL-5 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 strips to be used and put any remaining strips with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4 °C.
2. Pipet 50 µL of standard diluent or Tissue Culture Media into the S0 (0 pg/mL standard) wells.
3. Pipet 50 µL of Standards #1 through #5 into the appropriate wells.
4. Pipet 50 µL of the Samples into the appropriate wells.
5. Tap the plate gently to mix the contents, and seal with the plate sealer provided.
6. Incubate the plate at room temperature for 1 hour. Do not wash or decant the plate.
7. Pipet 50 µL of the Biotinylated Antibody into each well, except the Blank.
8. Seal the plate and incubate at room temperature for 1 hour.
9. 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.
10. Add 100 µL of the freshly prepared Streptavidin-HRP Conjugate to each well, except the Blank.
11. Seal the plate and incubate at room temperature for 30 minutes.
12. 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.
13. Pipet 100 µL of Substrate Solution into each well.
14. Incubate for 30 minutes at room temperature.
15. Pipet 100 µL Stop Solution 2 to each well.
16. 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-5 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-5 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.

   Average Net OD = Average OD - Average Blank OD

2. Using linear graph paper, plot the Average Net OD for each standard versus concentration of human IL-5 in each standard. Approximate a straight line through the points. The concentration of h IL-5 in the unknowns can be determined by interpolation.

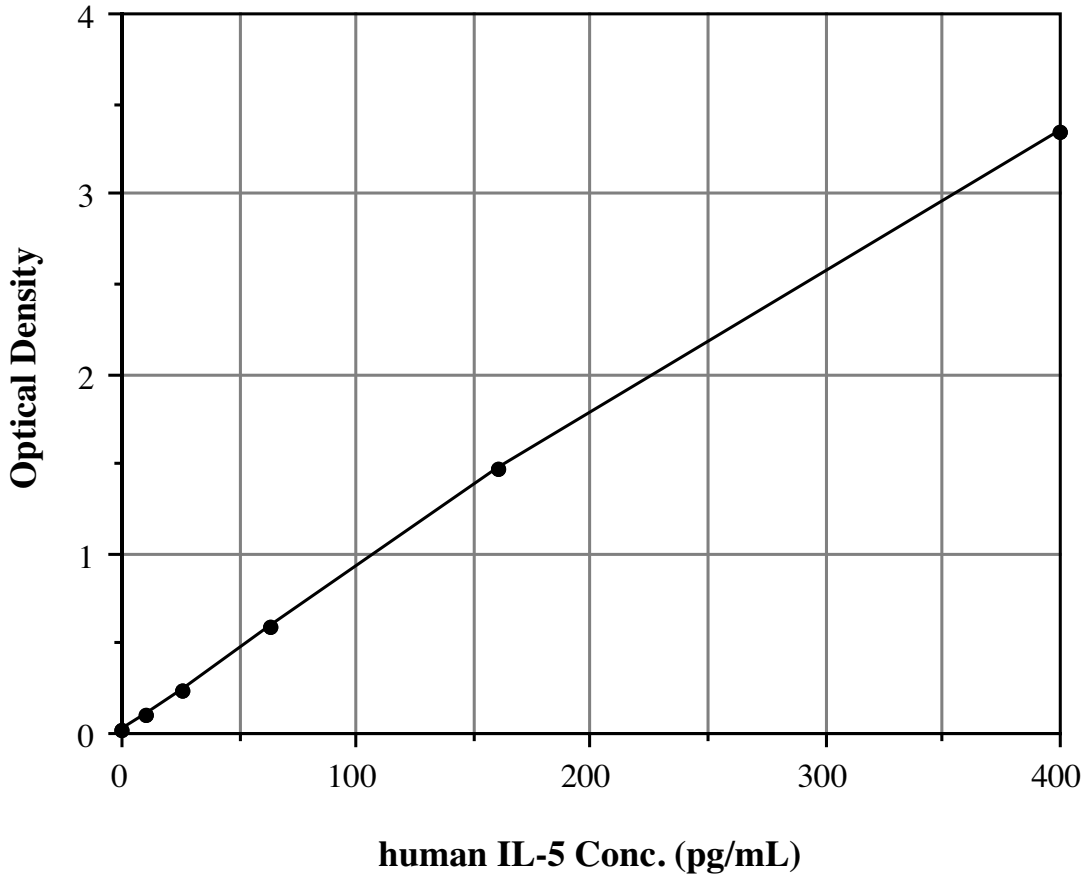
Typical Results

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

Sample	Average OD	Net OD	**h IL-5 (pg/mL)**
Blank	(0.044)		
S0	0.065	0.021	**0**
S1	3.388	3.344	**400**
S2	1.505	1.461	**160**
S3	0.633	0.589	**64**
S4	0.274	0.230	**25.6**
S5	0.139	0.095	**10.24**
Unknown 1	1.813	1.769	**195.2**
Unknown 2	0.299	0.255	**28.2**

Typical Standard Curve

A typical standard curve is shown below. This curve **must not** be used to calculate human IL-5 concentrations; each user must run a standard curve for each assay.



Performance Characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[10].

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

A sample containing 310.1 pg/mL human IL-5 was serially diluted 4 times 1:2 in the standard diluent supplied in the kit and measured in the assay. The data was plotted graphically as actual human IL-5 concentration versus measured human IL-5 concentration.

The line obtained had a slope of 0.932 with a correlation coefficient of 0.999.

Precision

Intra-assay CV: < 10%
Inter-assay CV: < 10%

Cross Reactivities

The TiterZyme® human IL-5 EIA Kit is specific for natural and recombinant human IL-5. It is unaffected by the presence of human IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, TNF-α, IFN-γ, IFN-α, GM-CSF, GROα, GROβ, or mouse IL-5.

Sample Recoveries

Cytokine recovery is determined by spiking various levels of recombinant human IL-5 in to serum, plasma, and urine samples collected from healthy individuals, and a Standard Diluent control buffer. Mean recoveries are as follows:

Spike Level	100 pg/mL	200 pg/mL	300 pg/mL
Serum (n=7)	100%	91%	92%
Plasma (n=7)	93%	85%	89%
Urine (n=8)	108%	n/d	n/d

References

1. T. Chard, "An Introduction to Radioimmunoassay & Related Techniques 4th Ed.", (1990) Amsterdam: Elsevier.
2. P. Tijssen, "Practice & Theory of Enzyme Immunoassays", (1985) Amsterdam: Elsevier.
3. A. McKenzie, et al., EMBO J., (1991) 10:1193.
4. C. Sanderson, "The Cytokine Handbook 3rd Ed.", (1998) San Diego: Academic Press.
5. W. Sewell and H. Mu, Immunol. Cell Biol., (1996) 74:274.
6. H. Kita et al., J. Allergy Clin. Imunol., (2000) 106(3 Pt 1):521.
7. N. Kenyon et al., Ann. Allergy Asthma Immunol., (2000) 85(2):115.
8. S. Imoto, Int. J. Hematol., (2000) 72(1):92.
9. C. Azuma et al., Nucleic Acids Res., (1986) 14(22):9149.
10. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

LIMITED WARRANTY

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

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