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TiterZyme® EIA

mouse IL-5

Enzyme Immunometric Assay Kit

Catalog No. 900-128

96 Well Kit

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Description

Assay Designs' mouse IL-5 TiterZyme® Enzyme Immunometric Assay (EIA) kit is a complete kit for the quantitative determintation of mouse IL-5 in biological fluids. Please read the complete kit insert before performing this assay. The kit uses a monoclonal antibody to mouse IL-5 immobilized on a microtiter plate to bind the mouse IL-5 in the standards or sample. A recombinant mouse IL-5 Standard is provided in the kit. After a short incubation the excess sample or standard is washed out and a Horseradish Peroxidase Conjugated antibody to mouse IL-5 is added. This antibody binds to the mouse IL-5 captured on the plate. After a short incubation the excess antibody 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 mouse 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]. While initially identified by a number of groups based on different biochemical aspects, IL-5 is now known to be the predominant cytokine in eosinophilia 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.

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 mouse IL-5 Standard provided, Catalog No. 80-1210, should be handled with care because of the known and unknown effects of IL-5.

Materials Supplied

1. **mouse IL-5 Microtiter Plate, One Plate of 96 Wells, Catalog No. 80-1209**
   A strip microtiter plate coated with antibody specific to mouse IL-5.
2. **Anti-mouse IL-5 Peroxidase Conjugate Reagent, 12 mL, Catalog No. 80-1213**
   A solution of antibody to mouse IL-5 conjugated with Horseradish Peroxidase.
3. **Standard Diluent, 12 mL, Catalog No. 80-1211**
   Phosphate buffered saline containing protein and 0.1% azide.
4. **Plate Reagent, 8 mL, Catalog No. 80-1212**
   Phosphate buffered saline containing protein and 0.1% azide.
5. **Wash Buffer Concentrate, 30 mL, Catalog No. 80-1286**
   Tris buffered saline containing detergents.
6. **mouse IL-5 Standard, 2 vials, Catalog No. 80-1210**
   Two vials containing lyophilized recombinant mouse IL-5.
7. **TMB Substrate, 11 mL, Catalog No. 80-0350**
   A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide. Ready to use. **Protect from prolonged exposure to light.**
8. **Stop Solution 2, 11 mL, Catalog No. 80-0377**
   A 1 N solution of hydrochloric acid. Keep tightly capped. Caution: **Caustic.**
9. **mouse IL-5 Assay Layout Sheet, 1 each, Catalog No. 30-0204**
10. **Plate Sealer, 3 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 µL and 1,000 µL.
3. Disposable polypropylene or polyethylene test tubes for dilution of samples and standards. Do not use polystyrene, polycarbonate or glass test tubes.
4. Repeater pipets for dispensing 50 µL.
5. Disposable beakers for diluting buffer concentrates.
6. Graduated cylinders.
7. A 37 °C incubator.
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 mouse IL-5 samples in mouse serum and Tissue Culture Media. Samples diluted sufficiently into the proper diluent can be read directly from a standard curve. Please refer to the Sample Recovery recommendations on page 11 for details of suggested dilutions.

Culture fluids and serum 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 mouse IL-5 in the appropriate matrix.

Samples must be stored frozen to avoid loss of bioactive mouse 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 -70 °C to avoid loss of bioactive mouse IL-5. 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 made up in either 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 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. Mouse IL-5 samples diluted with Standard Diluent should be run with a standard curve diluted in the same buffer. Serum 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 4 °C until the kit expiration, or for 3 months, whichever is earlier.

2. mouse IL-5 Standards

Reconstitution volume is stated on the standard vial label. If using serum samples, reconstitute standard with deionized water. If using tissue culture supernatants, reconstitute standard with tissue culture media. Let it stand for 5 minutes at room temperature. Mix gently by inverting the vial. Label the vial standard #1.

Label 2 12x75 mm test tubes #2 and #3. If using serum samples, pipet 600 µL of Standard Diluent into tubes #2 and #3. If using tissue culture supernatants, pipet 600 µL of tissue culture media in to tubes #2 and #3. Pipet 200 µL of reconstituted standard #1 into tube #2 and mix. Add 200 µL of tube #2 to tube #3 and mix.

The concentration of mouse IL-5 in standard vial #1 and tubes #2 and #3 will be 320, 80, and 20 pg/mL respectively. See mouse IL-5 Assay Layout Sheet for dilution details.

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 provided bag and seal the ziploc. Store unused strips at 4 °C.
2. Pipet 50 µL of Plate Reagent to all wells.
3. Pipet 50 µL of Standards #1 through #3 into the appropriate wells.
4. Pipet 50 µL of the Samples into the appropriate wells.
5. Tap the plate gently to mix the contents.
6. Pipet 50 µL of Standard Diluent to all wells that do not contain standards or samples.
7. Seal plate with plate sealer.
8. Incubate at 37 °C for 2 hours.
9. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 4 more times for a total of **5 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. Pipet 100 µL of Conjugate Reagent into each well.
11. Seal the plate and incubate at 37 °C for 1 hour.
12. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 4 more times for a total of **5 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, uncovered.
15. Pipet 100 µL Stop Solution 2 to each well. This stops the reaction and the plate should be read immediately.
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 mouse 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 mouse 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 mouse IL-5 concentration in each standard. Approximate a straight line through the points. The concentration of mouse IL-5 in the unknowns can be determined by interpolation.

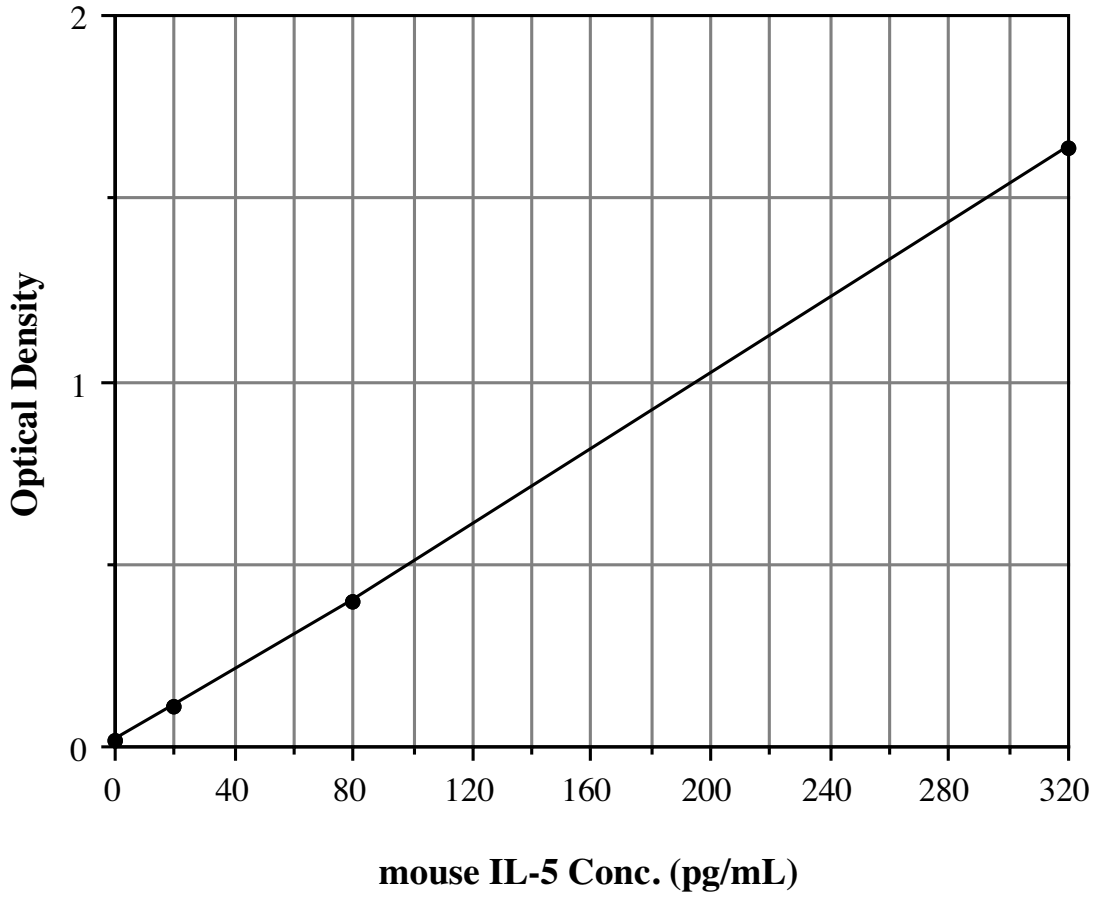
Typical Results

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

Sample	Average OD	Net OD	**m IL-5 (pg/mL)**
Blank	0.051		
S0	0.065	0.014	**0**
S1	1.686	1.635	**320**
S2	0.447	0.396	**80**
S3	0.163	0.112	**20**
Unknown 1	0.393	0.342	**68.6**
Unknown 2	1.264	1.213	**239.3**

Typical Standard Curve

A typical standard curve is shown below. This curve **must not** be used to calculate mouse 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

< 5 pg/mL.

Linearity

A sample containing 284.0 pg/mL mouse IL-5 was serially diluted 3 times 1:2 in the standard diluent supplied in the kit and measured in the assay. The data was plotted graphically as actual mouse IL-5 concentration versus measured mouse IL-5 concentration.

The line obtained had a slope of 1.00 with a correlation coefficient of 0.999.

Precision

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

Cross Reactivities

The TiterZyme® mouse IL-5 EIA Kit is specific for natural and recombinant mouse IL-5. It is unaffected by the presence of mouse IL-2, IL-3, IL-4, IL-6, IFNγ, GM-CSF, TNFα, rat IL-5, rabbit IL-5, or human IL-5.

Sample Recoveries

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

Mouse IL-5 concentrations were measured in mouse serum. Mouse IL-5 was spiked into the undiluted sample and compared with a spiked PBS/BSA control. The following results were obtained:

Sample	% Recovery *	Recommended Dilution *
Mouse Serum	83.5	None

* See Sample Handling instructions on page 4 for details.

References

1. T. Chard, "An Introduction to Radioimmunoassay & Related Techniques 4th Ed.", (1990) Amsterdam: Elsevier.
2. P. Tijssen, in "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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