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TiterZyme® EIA

mouse IL-10

Enzyme Immunometric Assay Kit

Catalog No. 900-148

96 Well Kit

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Description

Assay Designs' mouse IL-10 TiterZyme® Enzyme Immunometric Assay (EIA) kit is a complete kit for the quantitative determintation of mouse IL-10 in biological fluids. Please read the complete kit insert before performing this assay. The kit uses an antibody to mouse IL-10 immobilized on a microtiter plate to bind the mouse IL-10 in the standards or sample. A recombinant mouse IL-10 Standard is provided in the kit. After a short incubation, the excess sample or standard is washed out and a biotinylated antibody to mouse IL-10 is added. This antibody binds to the mouse IL-10 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 mouse IL-10 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 mouse IL-10 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-10 (IL-10) was initially identified as the 'Cytokine Synthesis Inhibitory Factor' based on the observation that mouse Th1 cytokine production was suppressed by a Th2-derived factor.[3] IL-10 is a macrophage deactivating factor, acting on macrophage-monocyte accessory cells to produce its inhibitory effects on T cells and natural killer cells. It also regulates growth and/or differentiation of B cells, mast cells, granulocytes, dendritic cells, keratinocytes and endothelial cells.[4] The ability of IL-10 to suppress Th1 activities while stimulating Th2 and humoral immune responses has been circumvented by a variety intracellular pathogens known to target macrophages. A number of parasites, bacteria, fungi and viruses depress host immune responses by either inducing host IL-10 production or encode their own IL-10 homologue.[5] Besides being a potent immunosuppressant, IL-10 is also an antipyretic and functions in mast cell homeostasis.[6,7] Circulating levels of IL-10 are increased in allergic asthma, systemic sclerosis, a variety of cancers, post-transplantation patients, and in sepsis.[8-12] The therapeutic potential of IL-10 includes rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and HIV infections.[13-17]

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 mouse IL-10 Standard provided, Catalog No. 80-1431, should be handled with care because of the known and unknown effects of IL-10.

Materials Supplied

1. **mouse IL-10 Microtiter Plate, One Plate of 96 Wells, Catalog No. 80-1427**
   A plate using break-apart strips coated with antibody specific to mouse IL-10.
2. **mouse IL-10 Antibody, 8 mL, Catalog No. 80-1428**
   A solution of biotinylated antibody to mouse IL-10.
3. **mouse IL-10 Assay Buffer, 12 mL, Catalog No. 80-1434**
4. **mouse IL-10 Streptavidin-HRP Concentrate, 75 µL, Catalog No. 80-1429**
   A concentrated solution of Streptavidin conjugated to Horseradish peroxidase.
5. **mouse IL-10 Streptavidin-HRP Dilution Buffer, 14 mL, Catalog No. 80-1430**
6. **Wash Buffer Concentrate, 50 mL, Catalog No. 80-1253**
   Tris buffered saline containing detergents.
7. **mouse IL-10 Standard, 2 vials, Catalog No. 80-1431**
   Two vials of lyophilized recombinant mouse IL-10.
8. **mouse IL-10 TMB Substrate, 13 mL, Catalog No. 80-1432**
   A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide. Ready to use. **Protect from prolonged exposure to light.**
9. **mouse IL-10 Stop Solution, 14 mL, Catalog No. 80-1433**
   A 0.18 M solution of sulfuric acid in water. Keep tightly capped. Caution: **Caustic.**
10. **mouse IL-10 Assay Layout Sheet, 1 each, Catalog No. 30-0226**
11. **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. No difference in assay results is seen with 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.
4. Repeater pipets for dispensing 50 µL and 100 µL.
5. Disposable beakers for diluting buffer concentrates.
6. Graduated cylinders.
7. Microcentrifuge to prepare Streptavidin-HRP solution.
8. Absorbent 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-10 samples in serum and tissue culture media. Samples can be read directly from a standard curve diluted in the proper diluent.

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 Assay Buffer. 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-10 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 mouse IL-10. 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. Mouse IL-10 samples diluted with Assay Buffer should be run with a standard curve diluted in the same buffer. Serum samples should be evaluated against a standard curve run in Assay Buffer 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. mouse IL-10 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 9,000 pg/mL mouse IL-10. When testing serum samples, use the Assay Buffer 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 400 µL of Assay Buffer or tissue culture media into tubes #1 through #5. Add 200 µL of the 9,000 pg/mL Standard to tube #1. Vortex thoroughly. Add 200 µL of tube #1 to tube #2 and vortex thoroughly. Add 200 µL of tube #2 to tube #3 and vortex thorougly. Continue this for #4 through #5.

The concentration of mouse IL-10 in tubes #1 through #5 will be 3,000, 1,000, 333, 111 and 37 pg/mL respectively. See mouse IL-10 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 100 µL of Assay Buffer or Tissue Culture Media into the S0 (0 pg/mL standard) wells.
3. Pipet 50 µL of Assay Buffer or Tissue Culture Media into the Standard and Sample 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 3 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 50 µL of Antibody into each well, except the Blank.
10. Seal the plate and incubate at room temperature for 1 hour.
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. Add 100 µL of Conjugate to each well, except the Blank.
13. Seal the plate and incubate at room temperature for 30 minutes.
14. 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.
15. Pipet 100 µL of Substrate Solution into each well.
16. Incubate for 30 minutes at room temperature in the dark.
17. Pipet 100 µL Stop Solution to each well. This stops the reaction and the plate should be read immediately.
18. 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-10 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-10 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 OD for each standard versus mouse IL-10 concentration in each standard. Approximate a straight line through the points. The concentration of mouse IL-10 in the unknowns can be determined by interpolation.

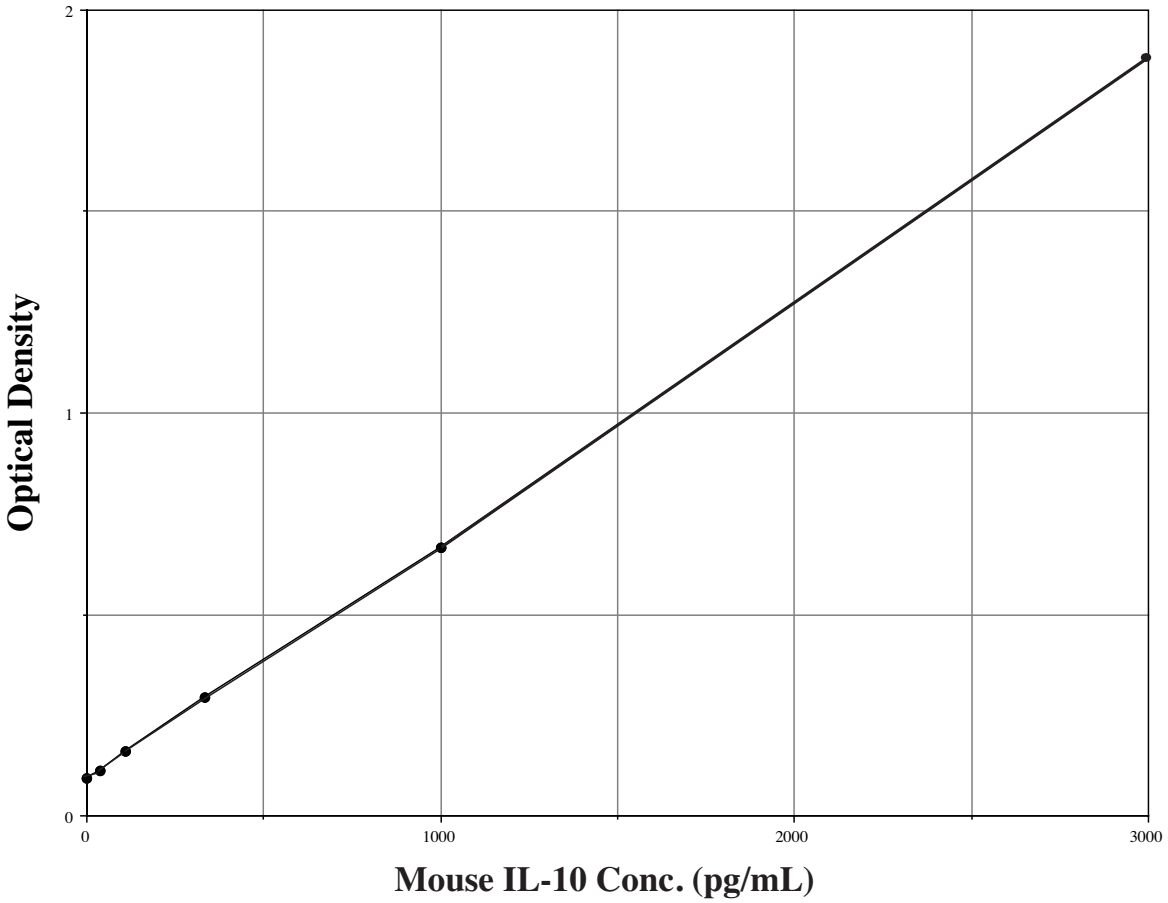
Typical Results

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

Sample	Average OD	**mouse IL-10 (pg/mL)**
S0	0.094	**0**
S1	1.876	**3,000**
S2	0.664	**1,000**
S3	0.292	**333**
S4	0.158	**111**
S5	0.111	**37**

Typical Standard Curve

A typical standard curve is shown below. This curve **must not** be used to calculate mouse IL-10 concentrations; each user must run a standard curve for each assay.



Performance Characteristics

Sensitivity <12 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:

Dilution linearity was determined by serially diluting 10 different positive samples. The dilutions were evaluated in the assay and "found" doses are plotted agains the "expected" doses. An "R" value and a slope of the regression line close to 1 indicate that the samples dilute linearly.

The line obtained had a slope of 0.917 with a correlation coefficient of 0.970.

Cross Reactivities

The mouse IL-10 TiterZyme® EIA Kit is specific for native and recombinant mouse IL-10. It is unaffected by the presence of mouse IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, GM-CSF, IFNγ, TNFα, or human IL-10.

Sample Recoveries

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

Recovery was determined by spiking three different levels of recombinant mouse IL-10 into eight serum samples collected from apparantly healthy Balb/c and NSA mice.
Recoveries are as follows:

Spike Level	Mean Recovery
100 pg/mL	116%
500 pg/mL	102%
1,500 pg/mL	101%

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

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