TiterZyme® EIA

mouse IL-2

Enzyme Immunometric Assay Kit

Catalog No. 900-042

96 Well Kit

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Description

Assay Designs' mouse IL-2 TiterZyme® Enzyme Immunometric Assay (EIA) kit is a complete kit for the quantitative determintation of mouse IL-2 in biological fluids. Please read the complete kit insert before performing this assay. The kit uses a monoclonal antibody to mouse IL-2 immobilized on a microtiter plate to bind the mouse IL-2 in the standards or sample. A recombinant mouse IL-2 Standard is provided in the kit. After a short incubation the excess sample or standard is washed out and a biotinylated polyclonal antibody to mouse IL-2 is added. This antibody binds to the mouse IL-2 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-2 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-2 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

Mouse IL-2 is a glycoprotein of 169 amino acids which is produced by activated T cells.[4,8] It has been shown to have effects on growth and differentiation of B and T cells, NK cells, LAK cells, monocytes, macrophages and oligodendrocytes.[4] It is also known as blastogenic factor (BF), eosinophil differentiation factor (EDF), TMF (thymocyte mitogenic factor), TMF (T-cell maturation factor), TMF (T-cell mitogenic factor), TRF-3 (T-cell replacing factor-3) and TSF (thymocyte stimulating factor).[7] IL-2 is mediated by its binding with the high-affinity IL-2 receptor.[4] This interaction causes activated T-cell expansion and proliferation while the resulting decline in IL-2 synthesis causes the cessation of the T-cell immune response.[3,4] IL-2 has also been shown to mediate immune response and promotes the production of IFN-γ, IL-1, TNF-α and TNF-β.[5,6,7] Human and mouse IL-2 share a 65% homology at the amino acid level.[8]

Precautions

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1. Stop Solution 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-2 Standard provided, Catalog No. 80-0626, should be handled with care because of the known and unknown effects of IL-2.
5. The mouse IL-2 Standard should be stored at or below -20°C . Do not repeatedly freeze-thaw.

Materials Supplied

1. **mouse IL-2 Microtiter Plate, One Plate of 96 Wells, Catalog No. 80-0343**
   A plate using break-apart strips coated with monoclonal antibody specific to mouse IL-2.
2. **mouse IL-2 Antibody, 6 mL, Catalog No. 80-0345**
   A yellow solution of biotinylated rat polyclonal antibody to mouse IL-2.
3. **Assay Buffer 13, 50 mL, Catalog No. 80-0863**
   Tris buffered saline containing proteins and detergents.
4. **Cytokine Conjugate 7, 6 mL, Catalog No. 80-0895**
   A blue solution of Streptavidin conjugated to Horseradish peroxidase.
5. **Wash Buffer Concentrate, 100 mL, Catalog No. 80-1287**
   Tris buffered saline containing detergents.
6. **mouse IL-2 Standard, 0.5 mL, Catalog No. 80-0626**
   A solution of 10,000 pg/mL mouse IL-2.
   Avoid repeated freeze/thaw cycles.
7. **TMB Substrate, 6 mL, Catalog No. 80-0615**
   A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide. Ready to use.
   Protect from prolonged exposure to light.
8. **Stop Solution, 11 mL, Catalog No. 80-0377**
   A 1N solution of hydrochloric acid in water. Keep tightly capped. Caution: **Caustic.**
9. **mouse IL-2 Assay Layout Sheet, 1 each, Catalog No. 30-0118**
10. **Plate Sealer, 3 each, Catalog No. 30-0012**

Storage

All components of this kit, **except the Standard**, are stable at 4˚C until the kit's expiration date. The Standard **must** be stored at or below -20˚C.

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Precision pipets for volumes between 50 µL and 1,000 µL.
3. Disposable test tubes for dilution of samples and standards.
4. Repeater pipets for dispensing 50 µL.
5. Disposable beakers for diluting buffer concentrates.
6. Graduated cylinders.
7. A microplate shaker.
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-2 samples in a wide range of matrices. 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, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the tissue culture media instead of Assay Buffer 13. 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-2 in the appropriate matrix.

Samples must be stored frozen to avoid loss of bioactive mouse IL-2. 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-2. 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 can be made up in either glass or plastic 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 break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed foil bag. The wells 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-2 samples diluted with Assay Buffer 13 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 13, 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 950 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-2 Standards

Allow the 10,000 pg/mL mouse IL-2 standard solution to warm to room temperature. Label eight 12 x 75 mm glass tubes #1 through #8. Pipet 900 µL of standard diluent (Assay Buffer 13 or tissue culture media) into tube #1. Pipet 500 µL of standard diluent into tubes #2 through #8. Add 100 µL of the 10,000 pg/mL standard to tube #1. Vortex thoroughly. Add 500 µL of tube #1 to tube #2 and vortex thoroughly. Add 500 µL of tube #2 to tube #3 and vortex. Continue this for tubes #4 through #8.

The concentration of mouse IL-2 in tubes #1 through #8 will be 1,000, 500, 250, 125, 62.5, 31.25, 15.63 and 7.81 pg/mL respectively. See mouse IL-2 Assay Layout Sheet for dilution details. STORE STANDARD AT -20˚C, avoid repeated freeze-thaws.

Assay Procedure

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

Plates require shaking on a microplate shaker at 500 rpm.

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 wells0with the desiccant back into the foil pouch and seal the ziploc. Store unused wells at 4˚C.
2. Pipet 50 µL of standard diluent (Assay Buffer 13 or tissue culture media) into the S0 (0 pg/mL standard) wells.
3. Pipet 50 µL of Standards #1 through #8 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. Seal the plate and incubate at room temperature, shaking, for 2 hours.
7. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 3 more times for a total of **4 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.
8. Pipet 50 µL of yellow Antibody into each well, except the Blank.
9. Seal the plate and incubate at room temperature, shaking, for 1 hour.
10. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 3 more times for a total of **4 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.
11. Add 50 µL of blue Conjugate to each well, except the Blank.
12. Seal the plate and incubate at room temperature, shaking, for 30 minutes.
13. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 3 more times for a total of **4 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.
14. Pipet 50 µL of Substrate Solution into each well.
15. Incubate at room temperature, shaking, for 15 minutes.
16. Pipet 50 µL Stop Solution to each well.
17. 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-2 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-2 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-2 concentration in each standard. Approximate a straight line through the points. The concentration of mouse IL-2 in the unknowns can be determined by interpolation.

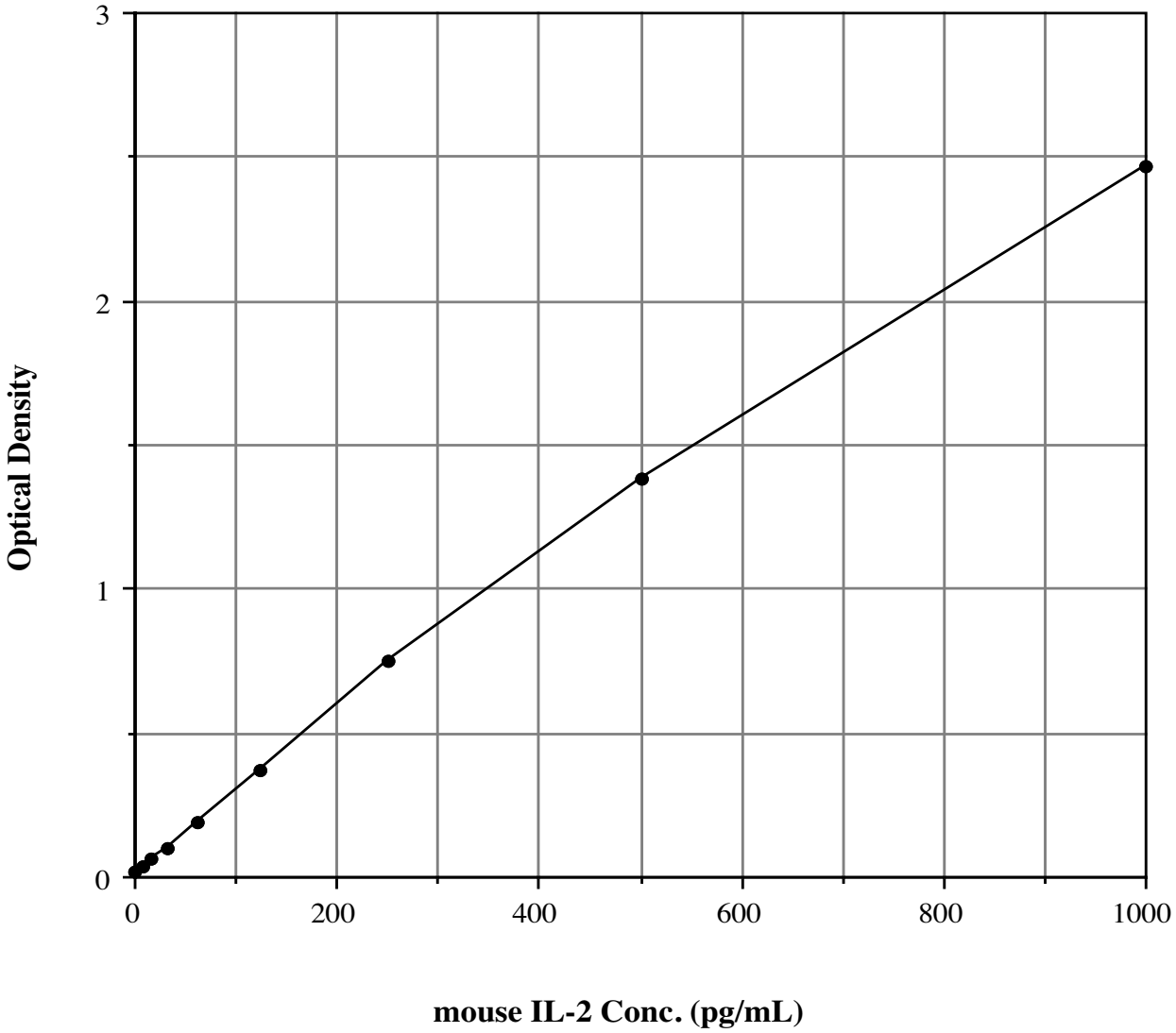
Typical Results

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-2 (pg/mL)**
Blank	(0.055)		
S0	0.072	0.017	**0**
S1	2.525	2.470	**1,000**
S2	1.439	1.384	**500**
S3	0.803	0.748	**250**
S4	0.422	0.367	**125**
S5	0.240	0.185	**62.5**
S6	0.156	0.101	**31.25**
S7	0.113	0.058	**15.63**
S8	0.091	0.036	**7.81**
Unknown #1	0.190	1.135	**44.2**
Unknown #2	1.397	1.342	**483.6**

Typical Standard Curve

A typical standard curve is shown below. This curve **must not** be used to calculate mouse IL-2 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[9].

Sensitivity

Sensitivity was calculated by determining the average optical density bound for sixteen (16) wells run with 0 pg/mL Standard, and comparing to the average optical density for sixteen (16) wells run with Standard #8. The detection limit was determined as the concentration of mouse IL-2 measured at two (2) standard deviations from the 0 pg/mL Standard along the standard curve.

Mean OD for S0 =	0.004 ± 0.002 (45.7%)
Mean OD for Standard #8 =	0.014 ± 0.002 (12.3%)
Delta Optical Density = (7.81 - 0 pg/mL) = 0.014 - 0.004 =	0.010
2 SD's of 0 pg/mL Standard = 2 x 0.002 =	0.004

$$\text{Sensitivity} = \frac{0.004}{0.010} \times 7.81 \text{ pg/mL} = \textbf{3.12 pg/mL}$$

Linearity

A sample containing 741.8 pg/mL mouse IL-2 was serially diluted 5 times 1:2 in the Assay Buffer 13 supplied in the kit and measured in the assay. The data was plotted graphically as actual mouse IL-2 concentration versus measured mouse IL-2 concentration.

The line obtained had a slope of 1.048 with a correlation coefficient of 0.9999.

Precision

Intra-assay precision was determined by taking samples containging low, medium and high concentrations of mouse IL-2 and running these samples multiple times (n=16) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of mouse IL-2 in multiple assays (n=8).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of mouse IL-2 determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	m IL-2 (pg/mL)	Intra-assay % CV	Inter-assay % CV
Low	16.4	8.7	
Medium	39.3	5.2	
High	509.9	3.2	
Low	19.3		10.3
Medium	41.6		6.7
High	514.1		6.8

Cross Reactivities

The TiterZyme® mouse IL-2 EIA Kit is specific for bioactive mouse IL-2. It is unaffected by the presence of the following recombinant molecules: human IL-2, mouse IL-1α, IL-1ß, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IFN-γ, GM-CSF, TNF-α and rat TNF-α.

Sample Recoveries

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

Mouse IL-2 concentrations were measured in mouse serum and tissue culture media. Mouse IL-2 was spiked into the undiluted samples of these matrices which were then diluted with the appropriate diluent and assayed in the kit. The following results were obtained:

Sample	% Recovery *	Recommended Dilution *
mouse Serum	91.9	1:4
Tissue Culture Media	97.5	1:2

* See Sample Handling instructions on page 4 for details.

References

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9. National Committee for Clinical Laboratory Standards Evaluation Protocols, SCI, 1989, NCCLS, Villanova, PA, 19085.

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

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