CELL APPLICATIONS, INC.

Mouse IL-4 ELISA Kit

Cat. No. CL0405
96-wells

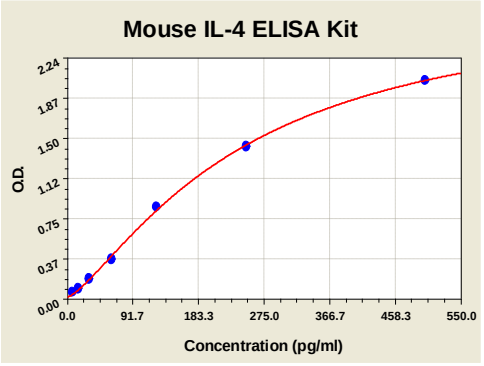
Target Protein Species:	Mouse
Range	7.8 pg/ml – 500pg/ml
Specificity	No detectable cross-reactivity with any other cytokines

KIT COMPONENTS

Component	Amount
96-well plate precoated with anti-mouse IL-4 antibody	1 Plate
Protein Standard: Lyophilized recombinant mouse IL-4	2 tubes, 10 ng/tube
Sample Diluent Buffer	30 ml
Biotinylated Antibody (Anti-mouse IL-4)	130 µl (100x)
Antibody Diluent Buffer	12ml
Avidin-Biotin-Peroxidase Complex (ABC) Solution	130 µl (100x)
ABC Diluent Buffer	12 ml
Tetramethylbenzidine (TMB) Color Developing Agent	10 ml
TMB Stop Solution	10 ml

Storage

Store at 4°C. Cell Applications, Inc. recommends using the kit within 6 months of order.



X	pg/ml	0.0	7.8	15.6	31.3	62.5	125	250	500
Y	O.D.450	0.005	0.065	0.105	0.198	0.375	0.862	1.4256	2.041

Figure 1: IL-4 Standard Curve. Using the mouse IL-4 ELISA Kit, O.D. data was graphed against IL-4 protein concentration. The TMB reaction was incubated at 37° C for 16 min.

BACKGROUND

Interleukin-4 (IL-4) is widely recognized as the canonical marker for Th2-polarized CD4+ T cells.[1] IL-4 was originally identified by its function as a B cell–stimulating factor, and numerous *in vivo* models of infection concur that IL-4 is critical for the isotype switch of B cells to IgE and IgG1. IL-4 also promotes the Th2 polarization of naive CD4+ T cells in vitro. However, in vivo studies have found that Th2 cells can develop in mice deficient for IL-4, the IL-4Ralpha chain, or the IL-4R–associated Stat 6. Stat6 has been shown to play a central role in mediating the immune regulatory signal of IL-4.[2] Collectively, these observations suggest that IL-4 production in sentinel lymph nodes draining a site of infection may be more important to support type 2 B cell responses rather than to establish the underlying Th2 response. Therefore, it stands to reason that the production of IL-4 by Th2 cells should preferentially occur in B cell follicles to optimize B cell help. In addition, IL-4 can prime macrophages to undergo additional, microbial-induced changes in cellular properties, relevant to host defense and pathogenesis of infectious and immune diseases.[3] IL-4 also regulates the expression of the low affinity Fc receptor for IgE (CD23) on both lymphocytes and monocytes.

Reference

1. Abbas, A.K. et al: Nature 383:787–793, 1996
2. van Panhuys, N. et al: Proc. Natl. Acad. Sci. USA. 105:12423–8, 2008
3. Varin, A. et al: Blood 115:353-62, 2010

ELISA OVERVIEW

Cell Applications ELISA Kits are based on standard sandwich enzyme-linked immunosorbent assay technology. Freshly prepared standards, samples, and solutions are recommended for best results.

1. Prepare test samples.
2. Prepare a protein standard of the target protein.
3. Add test samples and standards to the pre-coated 96-well plate. Do not wash.
4. Add biotinylated detection antibodies. Wash.
5. Add Avidin-Biotin-Peroxidase Complex (ABC) Solution. Wash.
6. Add Tetramethylbenzidine (TMB) Color Developing Agent, containing HRP substrate.
7. Add TMB Stop Solution
8. Subject the plate to analysis.

NOTES:

- Before using the kit, quick spin tubes to bring down all solution to the bottom of tube.
- Duplicate assay wells are recommended for both standard and sample testing.
- Do not let the 96-well plate dry, this will lead to inactivation of plate components.
- When diluting samples and reagents, ensure that they are mixed completely and evenly.
- Pre-warm diluted ABC and TMB solutions at 37°C for 30 min before use to avoid variable temperature effects.
- For washes, use TBS or PBS. Do not touch well walls.
- A protein standard is included in the kit. A protein standard detection curve should be generated with each experiment, no more than 2 hours prior to the experiment.
- The user will determine sample dilution fold by estimation of target protein amount in samples.

ELISA PROTOCOL

Preparation of Test Samples

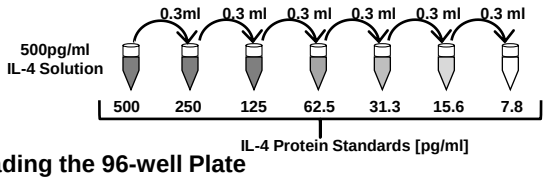
1. Process Test Samples in the following manner:
   - **Cell culture supernate, tissue lysate or body fluids**: Remove particulates by centrifugation.
   - **Serum**: Allow the serum to clot in a serum separator tube (about 30 min) at room temperature. Centrifuge at approximately 2000 x g for 20 min. Analyze the serum immediately or aliquot and store frozen at -20° C.
2. Estimate the concentration of the target protein in the sample and select a proper dilution factor such that the diluted target protein concentration falls within the 7.8-500 pg/ml standard curve range. Depending on the sample, several trial dilutions may be necessary. Dilute the sample using the provided diluent buffer, mixing well. Suggested working dilutions of samples are as follows:

Target Protein Concentration Range	Sample Working Dilution	Sample Vol.	Diluent Buffer Vol.
5-50 ng/ml	1:100	1 µl	99 µl
500-5000 pg/ml	1:10	10 µl	90 µl
7.8-500 pg/ml	1:2	50 µl	50 µl
≤7.8 pg/ml	n/a	100µl	n/a

3. If samples will be assayed within 24 hours, store at 2-8° C. For long-term storage, aliquot and freeze samples at -20° C. Avoid repeated freeze-thaw cycles.

Preparation of Standard Solutions (7.8-500 pg/ml)

4. Reconstitute the Lypophilized Recombinant Protein to make a 10.000 pg/ml mouse IL-4 solution. Add 1 ml Sample Diluent Buffer to a tube of lypophilized protein, keep the tube at room temperature for 10 min. Mix thoroughly.
5. Add 0.05 ml of the mixed 10,000 pg/ml IL-4 solution to the eppendorf tube containing 0.95 ml diluent buffer and mix to make a 500 pg/ml IL-4 solution.
6 Label 6 eppendorf tubes with the IL-4 protein concentrations to be prepared by serial dilution: 250pg/ml, 125pg/ml, 62.5pg/ml, 31.3pg/ml, 15.6pg/ml, 7.8pg/ml.
7. Aliquot 0.3 ml of the Sample Diluent Buffer to the labeled tubes.
8. Serially dilute the protein standards into their respectively labeled tubes. Transfer 0.3 ml from the 500pg/ml IL-4 Solution to the 250pg/ml eppendorf tube and mix thoroughly. Transfer 0.3 ml of the 250 pg/ml solution to the 125pg/ml tube and mix thoroughly, Transfer 0.3 ml of the 125pg/ml solution to the 62.5pg/ml tube and mix, and so on to make the 31.3, 15.6 and 7.8 pg/ml solutions.
9. Store at 4° C until use.



Loading the 96-well Plate

10. Aliquot 0.1 ml of the sample diluent buffer into a control well to serve as the Blank. This will yield the O.D.450(Blank) reading.
11. Aliquot 0.1 ml of the standard solutions of the **Preparation of Standard Solutions** (7.8-500pg/ml) into empty wells of the precoated 96-well plate. Duplicate measurements of standards are recommended.
12. Aliquot 0.1 ml of each properly diluted test sample to empty wells prepared in **Step 2**. Duplicate measurements of each test sample are recommended.
13. Cover the 96-well plate and incubate at 37° C for 90 min.
14. During the **Step 13** incubation period, prepare a stock of Biotinylated 1:100 Antibody Working Solution. Count the number of reactions and multiply by 0.1 ml/well for the Working Solution total volume (preparation of 1-2 reactions in excess of the number of wells is recommended). Dilute the Biotinylated Antibody to 1:100 in Antibody Diluent Buffer and mix thoroughly. Use the working solution within 2 hours.
15. Upon completion of the 90 min incubation of **Step 13**, remove the cover of the 96 well plate and discard plate well contents. Blot the plate onto paper towels or other absorbent material. DO NOT let the wells completely dry at any time.
16. Add 0.1 ml of the Biotinylated 1:100 Antibody Working Solution (prepared in **Step 14**) to each well and incubate the plate at 37° C for 60 min.
17. During the incubation period of **Step 16**, prepare a stock of ABC Working Solution. Count the number of reactions and multiply by 0.1 ml/well for the Working Solution total volume (preparation of 1-2 reactions in excess of the number of wells is recommended). Dilute the ABC Stock Solution to 1:100 in ABC Diluent Buffer and mix thoroughly. Pre-warm the ABC working solution at 37° C for 30 min before use. Use the working solution within 1 hour.
18. Upon completion of the 60 min incubation of **Step 16**, wash the plate 3 times with 0.3 ml TBS or PBS. For each wash, leave washing buffer in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
19. Add 0.1 ml of prepared ABC Working Solution (prepared in **Step 17**) to each well and incubate the plate at 37° C for 30 min.
20. During the incubation period of **Step 19**, pre-warm TMB Color Developing Agent at 37° C for 30 min before use.
21. Upon completion of the 30 min incubation of **Step 19**, wash the plate 5 times with 0.3 ml TBS or PBS. For each wash, leave the washing buffer in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
22. Add 90 µl of the pre-warmed TMB Color Developing Agent into each well and incubate at 37° C for 15-20 min (shades of blue can be seen in the wells with the four most concentrated Protein Standard Solutions; the other control wells should show no obvious color).
23. Add 0.1 ml of the TMB Stop Solution to each well. The acidic stop solution will change the mixture color to yellow. The yellow intensity is proportional to the amount of target protein captured by the plate.
24. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution. These readings are the O.D.450(Reading).

Calculating Protein Concentration

- For all wells, determine O.D.450(Relative):
  O.D.450(Relative) = O.D.450(Reading) – O.D.450(Blank)
- Plot the standard curve: plot O.D.450(Relative) of each standard solution (Y) vs. the respective concentration of the standard solution (X). See **Figure 1** for a typical standard curve.
- The mouse IL-4 concentration of the samples can be interpolated from the standard curve. Multiply the interpolated concentration by the dilution factor to obtain the target protein concentration in the sample.